

**Repository of the Max Delbrück Center for Molecular Medicine (MDC)
in the Helmholtz Association**

<http://edoc.mdc-berlin.de/15950>

**Identification of novel regulators of developmental hematopoiesis using
endoglin regulatory elements as molecular probes**

Nasrallah, R. and Fast, E.M. and Solaimani, P. and Knezevic, K. and Eliades, A. and Patel, R. and Thambyrajah, R. and Unnikrishnan, A. and Thoms, J. and Beck, D. and Vink, C.S. and Smith, A. and Wong, J. and Shepherd, M. and Kent, D. and Roychoudhuri, R. and Paul, F. and Klippert, J. and Hammes, A. and Willnow, T. and Goettgens, B. and Dzierzak, E. and Zon, L.I. and Lacaud, G. and Kouskoff, V. and Pimanda, J.E.

This is a copy of the final article, which was first published online on 23 AUGUST 2016 and in final edited form in:

Blood
2017 OCT 13 ; 128(15): 1928-1939
doi: [10.1182/blood-2016-02-697870](https://doi.org/10.1182/blood-2016-02-697870)

Publisher: [The American Society of Hematology](#)

HEMATOPOIESIS AND STEM CELLS

Identification of novel regulators of developmental hematopoiesis using *Endoglin* regulatory elements as molecular probes

Rabab Nasrallah,¹ Eva M. Fast,^{2,3} Parham Solaimani,⁴ Kathy Knezevic,¹ Alexia Eliades,⁵ Rahima Patel,⁵ Roshana Thambyrajah,⁵ Ashwin Unnikrishnan,¹ Julie Thoms,¹ Dominik Beck,¹ Chris S. Vink,^{4,6} Aileen Smith,⁷ Jason Wong,¹ Mairi Shepherd,⁷ David Kent,⁷ Rahul Roychoudhuri,⁸ Fabian Paul,⁹ Julia Klippert,⁹ Annette Hammes,⁹ Thomas Willnow,⁹ Bertie Göttgens,⁷ Elaine Dzierzak,^{4,6} Leonard I. Zon,^{2,3} George Lacaud,⁵ Valerie Kouskoff,⁵ and John E. Pimanda^{1,10}

¹Lowy Cancer Research Centre and the Prince of Wales Clinical School, University of New South Wales Australia, Sydney, Australia; ²Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA; ³Howard Hughes Medical Institute, Stem Cell Program and Division of Pediatric Hematology/Oncology, Boston Children's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; ⁴Erasmus Medical Center Stem Cell Institute, Department of Cell Biology, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁵Cancer Research United Kingdom Manchester Institute, The University of Manchester, Manchester, United Kingdom; ⁶Medical Research Council Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom; ⁷Department of Haematology, Wellcome Trust and Medical Research Council Cambridge Stem Cell Institute and Cambridge Institute for Medical Research, Cambridge University, Cambridge, United Kingdom; ⁸The Babraham Institute, Babraham Research Campus, Cambridge, United Kingdom; ⁹Max Delbrueck Center for Molecular Medicine, Berlin, Germany; and ¹⁰Department of Haematology, The Prince of Wales Hospital, Sydney, Australia

Key Points

- ENG regulatory elements target hemogenic mesoderm and hemogenic endothelium.
- Hemogenic progenitors can be enriched using these elements as molecular probes to discover novel regulators of hematopoiesis.

Enhancers are the primary determinants of cell identity, and specific promoter/enhancer combinations of *Endoglin* (ENG) have been shown to target blood and endothelium in the embryo. Here, we generated a series of embryonic stem cell lines, each targeted with reporter constructs driven by specific promoter/enhancer combinations of ENG, to evaluate their discriminative potential and value as molecular probes of the corresponding transcriptome. The *Eng* promoter (P) in combination with the $-8/+7/+9$ -kb enhancers, targeted cells in FLK1 mesoderm that were enriched for blast colony forming potential, whereas the $P/-8$ -kb enhancer targeted TIE2+/c-KIT+/CD41- endothelial cells that were enriched for hematopoietic potential. These fractions were isolated using reporter expression and their transcriptomes profiled by RNA-seq. There was high concordance between our signatures and those from embryos with defects at corresponding stages of hematopoiesis. Of the 6 genes that were upregulated in both hemogenic mesoderm and hemogenic endothelial fractions targeted by the reporters, LRP2, a multiligand receptor, was the only gene that had not previously been associated with hematopoiesis. We show that LRP2 is indeed involved in definitive hematopoiesis and by doing so validate the use of reporter gene-coupled enhancers as probes to gain insights into transcriptional changes that facilitate cell fate transitions. (*Blood*. 2016;128(15):1928-1939)

Introduction

With advances in microscopy and histology, different cell types can now readily be distinguished from one another. However, the molecular characteristics that make each cell type unique and help distinguish stem cells from their more differentiated progeny in a tissue are still obscure. Harvesting pure populations of stem cells is a prerequisite to probing their molecular identity. Over the years, protocols combining flow cytometry with single-cell serial transplantation assays have been progressively refined to purify mouse and human adult hematopoietic stem cells (HSCs).^{1,2}

One of the utilitarian benefits of determining the molecular fingerprint of an HSC is that it could serve as a measurable goal when developing protocols aimed at generating HSCs from differentiated cells.³ The failure of current protocols to generate long-

term repopulating HSCs from embryonic stem/induced pluripotent stem (ES/iPS) cells is attributed in part to our incomplete understanding of the developmental journey that mesodermal progenitors traverse in the embryo when generating the complement of HSCs that are resident in the bone marrow of a newborn.⁴ Determining the molecular identities of embryonic HSC precursors is complicated by the lack of consensus regarding the precise HSC intermediates in the embryo, functional assays that are less than ideal for assessment of these intermediates and knowledge that these intermediates are transitory cell populations that are present in very small numbers.⁵ FLK1 expressing mesodermal cells in the posterior primitive streak when isolated from the embryo and cultured in vitro generate blast colonies that have blood,

Submitted 3 February 2016; accepted 15 August 2016. Prepublished online as *Blood* First Edition paper, 23 August 2016; DOI 10.1182/blood-2016-02-697870.

The data reported in this article have been deposited in the Gene Expression Omnibus database (accession number GSE77390).

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2016 by The American Society of Hematology

endothelial, and vascular smooth muscle potential.⁶ Blast colony forming cell (BL-CFC) potential in FLK1+ mesoderm has been estimated to be ~1:300.⁷ Hemogenic potential in TIE2+c-KIT+ hemogenic endothelium (HE) or VE-CAD+CD45-CD41- pre-HSC cells in the dorsal aorta that transit to hematopoietic cells range from 1:100 to 1:300.⁸⁻¹⁰ These functional estimates are too low to probe the molecular identities of either the early hemangioblast or HE cell populations in the developing embryo using currently available protocols.

Cell identity is encoded within the sequences of tissue-specific gene regulatory elements (GREs) that direct and coordinate gene expression in a cell.¹¹ A number of regulatory elements of hematopoietic transcription factors (TFs) have previously been shown to direct reporter expression to developing blood cells in the mouse embryo and include enhancers of *Scl*, *Runx1*, *Gata2*, *Erg*, *Fli1*, *Lmo2*, and *Lyl1*, which also form a recursive circuit in human adult HSCs.¹² The *Runx1*+23 enhancer marks a population of early HE cells that transit to HSCs and has been used to isolate cells from different embryonic stages for transcriptomic analysis.¹³ *Ly6a/Scal* and *Eng* (CD105) serve as useful cell surface markers for isolation of murine HSC fractions.^{14,15} The promoter of *Ly6a* and promoter/enhancer combinations of *Eng* also target embryonic hematopoiesis and in the case of the former have been used in conjunction with a reporter to isolate HE cells and HSCs from early embryos.¹⁶⁻¹⁸

Endoglin (ENG) is an accessory receptor and modulator of TGF- β superfamily signaling.¹⁹ ENG is expressed on FLK1+ mesoderm and is required for normal BL-CFC development, and its expression facilitates the hematopoietic program in these cells.^{10,20} ENG null mice die at E9.5 with vascular defects due to abnormal endothelial and pericyte development.²¹ It is also a marker of adult murine HSCs that was identified using a *Scl*+19-driven fluorescent reporter coupled with transcriptomic and proteomic assessment of purified cells.¹⁵ An emerging concept of developmental hematopoiesis posits that HSC development from the dorsal aorta at E10 reflects maturation of cells that were fated earlier during embryogenesis toward the hematopoietic lineage.¹³ As such, we rationalized that transcriptional regulation of ENG, which is functionally important for the development of hematopoietic intermediates, could be instructive in helping elucidate the transcriptional environment of these cells. We have previously shown that sequence information within the promoter and hematoendothelial enhancers of *Eng* determine how reporter genes are targeted to either endothelial or blood and endothelial tissues in the embryo.^{17,22} Given the spectrum of cell types that are involved in the developmental journey of embryonic HSCs and the deterministic role that ENG plays in their development, we hypothesized that distinct combinations of promoter/enhancers of this gene are used by different hematopoietic intermediates to regulate *ENG* expression. We rationalized that if distinct promoter/enhancer constructs indeed targeted functionally distinct hematopoietic intermediates, they could be used as molecular probes to profile the transcriptional environment of these cells.

Here, we show using ES cells with single-copy reporter-coupled transgenes targeted to the constitutively active HPRT locus that distinct promoter/enhancer combinations of ENG are used by FLK1+ mesoderm and HE that are enriched for BL-CFC and hematopoietic potential, respectively. Using these reporter-coupled transgenes as probes to harvest cell populations from embryonic stem cell (ESC) differentiation assays, we performed RNA-seq to identify gene sets that were associated with functional enrichment of hematopoietic potential and show their complementarity with primary mouse tissues at matching stages of development.

Of the 6 genes that were upregulated in both hemogenic mesoderm (HB) and HE fractions targeted by the reporters, LRP2, a multiligand receptor, was the only gene that had not previously been associated with hematopoiesis. Here, we show that LRP2 is indeed involved in aorta-gonad-mesonephros (AGM) hematopoiesis and by doing so validate the use of reporter gene-coupled enhancers as a discovery tool.

Materials and methods

Murine ES cell culture and Hprt targeting

The Bry/GFP⁷ and HM1 ES cells²³ were cultured as previously described. See supplemental data, available on the *Blood* Web site, for description.

ES cell differentiation into embryoid bodies (EBs) and lacZ staining

To generate EBs, ES cells were collected and cultured as detailed in the supplemental data.

Flow cytometry and cell sorting

Cells were collected from EBs and liquid blast cultures and dissociated into a single-cell suspension. Details of the procedure and antibodies are listed in the supplemental data.

Methylcellulose blast BL-CFC assay and liquid cultures

Details of the BL-CFC assay and liquid cultures are listed in the supplemental data.

Hematopoietic methylcellulose colony-forming assay

Cells isolated from day 2 or 4 of liquid blast cultures and seeded for colony-forming unit-cell (CFU-C) assays as detailed in the supplemental data.

Reverse transcription polymerase chain reaction (RT-PCR)

A list of primers and methods are supplied in the supplemental data.

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed as detailed previously.²² See the supplemental data for a list of primers and experimental details.

Mouse embryo immunostaining and imaging

Details of the procedure and antibodies are listed in the supplemental data.

Generating zebrafish morpholinos and analysis

Details of generating zebrafish morpholinos and the analysis are listed in the supplemental data.

Statistical analysis

RT-PCR data, BL-CFCs, and hematopoietic colony counts were statistically analyzed using Student *t* test or paired Student *t* test.

RNA sequencing and analysis

The data have been deposited in the Gene Expression Omnibus under the accession number GSE77390. The Ingenuity IPA Core Analysis Tool (version 17199142) and the GSEA Java Desktop tool (v 2.0.13) were used for analysis. See the supplemental data for details.

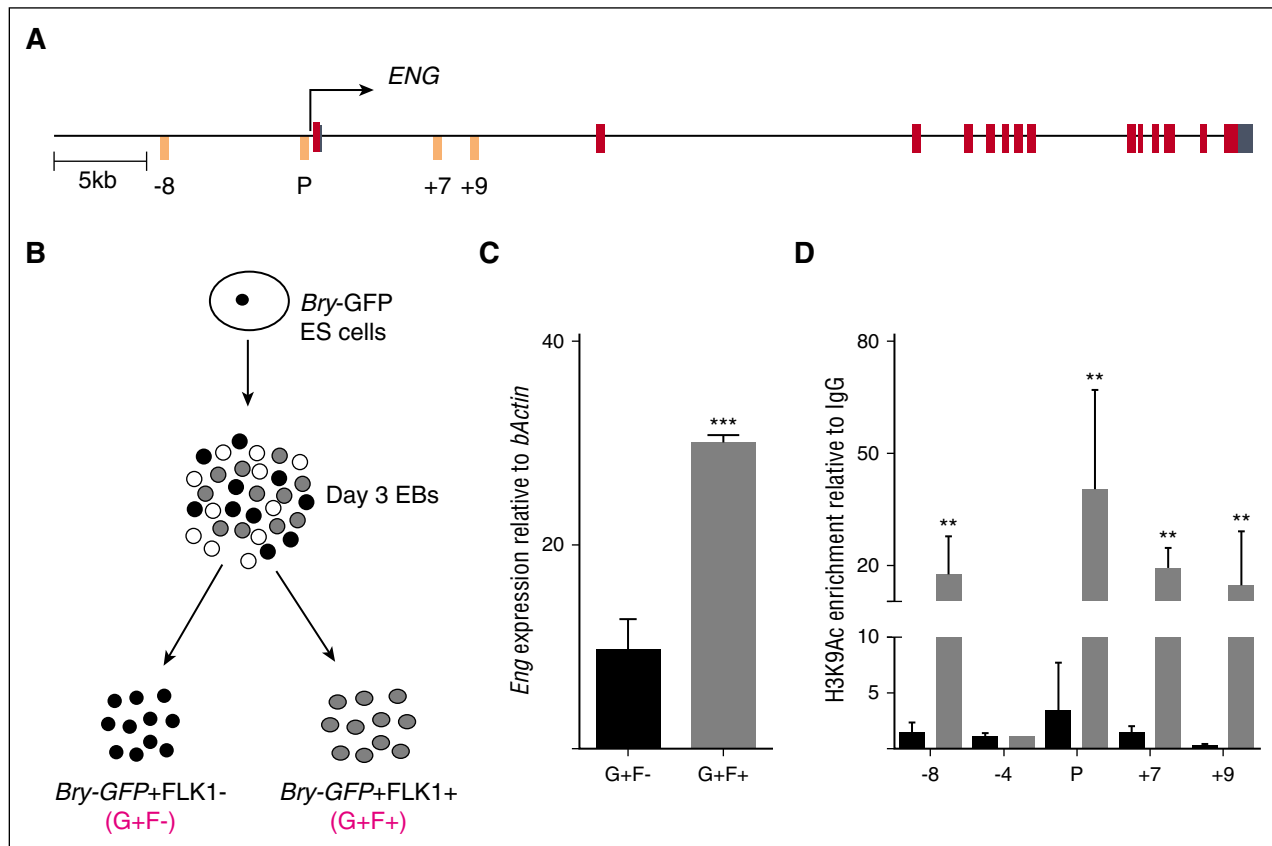


Figure 1. Mesoderm to hemangioblast transition is accompanied by increased *Eng* expression and chromatin accessibility at hematoendothelial regulatory elements. (A) Schematic representation of the *ENG* locus. The transcription start site is marked with an arrow. The -8 -kb, $+7$ -kb, and $+9$ -kb enhancers and the promoter (P) are marked in orange; exons are marked in brown, and the 5' untranslated region is marked in cyan. (B) Schematic representation of *Bry*-GFP ES cell differentiation. At day 3 of EB differentiation, *Bry*-GFP+/FLK1 $-$ (G+F $-$) and *Bry*-GFP+/FLK1 $+$ (G+F $+$) cells were sorted and analyzed by RT-PCR and ChIP. (C) Bar graph shows *Eng* mRNA expression levels in sorted FLK1 $+$ ve and $-$ ve mesodermal cell populations in day 3 EBs generated from *Bry*-GFP ES cells. (D) Bar graph shows levels of enrichment of the active chromatin mark, H3K9Ac at *Eng* -8 , P, $+7$, and $+9$ hematoendothelial enhancers relative to immunoglobulin G in prehemangioblast mesoderm (G+F $-$; black) and in hemangioblast mesoderm (G+F $+$; gray). *Eng* -4 was included as a negative control region. ** $P < .01$, *** $P < .001$.

Study approval

All experiments were approved by the institutional biological committee and animal care and ethics committee of the University of New South Wales, Manchester University, and Harvard University.

Results

Mesoderm to hemangioblast transition is accompanied by increased *Eng* expression and chromatin accessibility at hematoendothelial regulatory elements

The promoter of *ENG* when coupled with -8 -kb, $+7$ -kb, and $+9$ -kb enhancers have previously been shown to direct reporter expression to either endothelial or blood and endothelial tissues in the embryo (Figure 1A^{17,22}). The *Bry*-GFP ESC line has been used extensively to investigate the developmental progression of premesoderm (GFP $-$ /FLK1 $-$) to prehemangioblast mesoderm (GFP+/FLK1 $-$; G+F $-$) to the hemangioblast (GFP+/FLK1 $+$; G+F $+$)⁷ (Figure 1B). We used this cell line to first evaluate expression of *Eng* and chromatin accessibility at hematoendothelial regulatory elements of *Eng*^{17,22} because cells progressed from prehemangioblast mesoderm to hemangioblast mesoderm. *Eng* expression increased by ~ 3 -fold (Figure 1C) and enrichments of H3K9 acetylation (an active chromatin mark) increased ~ 10 - to 20-fold at the *Eng* promoter and -8 -kb, $+7$ -kb, and $+9$ -kb

Eng enhancers (Figure 1D). There was no change in H3K9Ac at -4 -kb, a region that is highly conserved across species but shows no enhancer activity.²²

The *Eng* promoter, when combined with the -8 , $+7$, and $+9$ hematoendothelial enhancers, targets FLK1 $+$ mesodermal cells enriched for BL-CFC potential

HM1 ESCs have a disrupted *Hprt* locus that can be reconstituted by homologous recombination of a targeting vector.²³ They serve as a useful tool to evaluate reporter activity of single copies of GREs at a constitutively active locus at different stages of ESC differentiation. We took advantage of this system to introduce combinations of *Eng* regulatory elements with blood and endothelial activity in in vivo transgenic assays (supplemental Figure 1). Successful recombination and generation of ESC lines with -8 /P/lacZ, -8 /P/lacZ/ $+7$, -8 /P/lacZ/ $+9$, -8 /P/lacZ/ $+7$ / $+9$, -8 /P/lacZ/ $+7$ Δ (GATA)/ $+9$, and -8 /P/lacZ/ $+7$ Δ (GATA)/ $+9$ Δ (ETS), were confirmed by RT-PCR and southern blotting (supplemental Figure 2). We used these ESC lines as a toolkit with which to track, evaluate, and compare the activity of each of these GREs during different stages of hematopoietic development and to fractionate cells for functional validation and transcriptomic analysis.

To identify which, if any, of the *Eng* GREs targeted FLK1 $+$ mesoderm enriched for hemangioblast potential, we generated EBs from each ESC line and fractionated FLK1 $+$ lacZ $-$ (F+L $-$) and FLK1 $+$ lacZ $+$ (F+L $+$) cells and performed BL-CFC assays

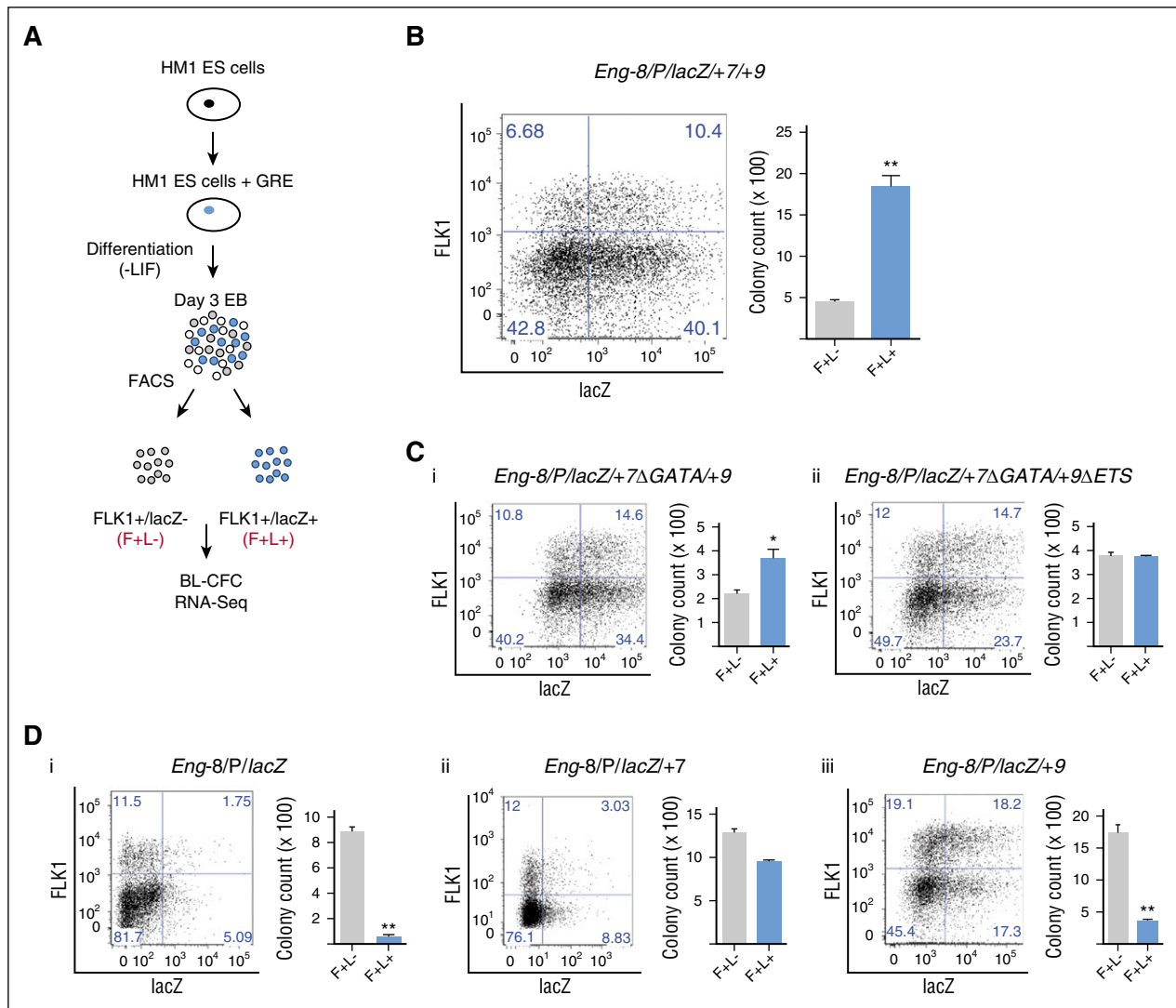


Figure 2. The *Eng* promoter when combined with the -8 , $+7$, and $+9$ hemoendothelial enhancers targets FLK1 $^{+}$ mesodermal cells enriched for BL-CFC potential. (A) Schematic representation of the experimental procedure. The *Eng* $-8P/LacZ$, *Eng* $-8P/LacZ/+7$, *Eng* $-8P/LacZ/+9$, *Eng* $-8P/LacZ/+7/+9$, *Eng* $-8P/LacZ/+7\Delta/+9$, and *Eng* $-8P/LacZ/+7\Delta/+9\Delta$ reporter constructs were introduced by homologous recombination into the HPRT locus of HM1 ES cells. Recombinant clones were differentiated into day 3 EBs and stained for FLK1 expression and β -galactosidase activity. FLK1 $^{+}/LacZ^{-}$ (F+L $^{-}$; gray) and FLK1 $^{+}/LacZ^{+}$ (F+L $^{+}$; blue) cells were sorted and seeded into BL-CFC assays. Fractions sorted from the *Eng* $-8P/LacZ/+7/+9$ were further analyzed by RNA sequencing. (B) Flow cytometry profiles of *Eng* $-8P/LacZ/+7/+9$ day 3 EBs (left). BL-CFCs from sorted F+L $^{-}$ (gray) and F+L $^{+}$ (blue) fractions. (C) (i) Flow cytometry profile of day 3 EBs derived from ES cells targeted with *Eng* $-8P/LacZ/+7\Delta/+9$ (mutated GATA motifs in the $+7$ enhancer) is shown to the left with corresponding BL-CFCs from sorted F+L $^{-}$ (gray) and F+L $^{+}$ (blue) fractions shown to the right. (ii) Flow cytometry profile of day 3 EBs derived from ES cells targeted with *Eng* $-8P/LacZ/+7\Delta/+9\Delta$ (mutated GATA motifs in the $+7$ enhancer and mutated ETS motifs in the $+9$ enhancer) and corresponding BL-CFCs from sorted F+L $^{-}$ (gray) and F+L $^{+}$ (blue) fractions. (D) Flow cytometry profiles of day 3 EBs and BL-CFCs from sorted F+L $^{-}$ (gray) and F+L $^{+}$ (blue) fractions are shown for ES cells targeted with (i) *Eng* $-8P/LacZ$, (ii) *Eng* $-8P/LacZ/+9$, and (iii) *Eng* $-8P/LacZ/+7$. BL-CFC counts are the total number of blast colonies generated from 2×10^4 seeded cells. Statistical analysis was conducted using Student *t* test, **P* < .05, ***P* < .01.

(Figure 2A). The *Eng* $-8P/lacZ/+7/+9$ construct, which showed robust blood and endothelial staining in vivo,¹⁷ targeted a fraction of the FLK1 $^{+}$ mesoderm that showed increased (~ 4 -fold) BL-CFC potential (Figure 2B; supplemental Figure 3A). We have previously shown that mutating the GATA binding motifs in $+7$ and ETS binding motifs in $+9$ diminished endothelial activity and extinguished hematopoietic activity of the *Eng* $-8P/lacZ/+7/+9$ construct in transgenic assays.¹⁷ There was a corresponding reduction or failure of the mutant constructs to preferentially target cells with BL-CFC potential (Figure 2Ci,ii; supplemental Figure 3B). The $-8P/lacZ$ construct showed strong endothelial but no hematopoietic activity in transgenic assays.¹⁷ FLK1 $^{+}$ cells targeted by this construct (F+L $^{+}$) showed significantly lower BL-CFC potential than F+L $^{-}$ cells (Figure 2Di; supplemental Figure 3A). For

the $-8P/lacZ/+7$ and $-8P/lacZ/+9$ constructs, which showed strong endothelial and low to moderate hematopoietic activity in in vivo transgenic assays,¹⁷ FLK1 $^{+}$ mesoderm (F+L $^{+}$) had either lower or equivalent BL-CFC activity than FLK1 $^{+}$ (F+L $^{-}$) cells (Figure 2Dii,iii; supplemental Figure 3A). It is important to note that the total number of BL-CFCs generated by FLK1 $^{+}$ mesoderm will vary from clone to clone but that comparisons are of BL-CFC potential of L $^{+}$ and L $^{-}$ sorted FLK1 $^{+}$ cells from each clone.

Taken together, these data show that the GREs of ENG that showed increased chromatin accessibility as prehemangioblast mesoderm progressed to hemangioblast mesoderm (ie, $-8P/+7/+9$; Figure 1D) act collectively to target reporter gene expression to BL-CFCs in FLK1 $^{+}$ mesoderm. It is noteworthy that in in vivo transgenic assays, it was also this construct ($-8P/lacZ/+7/+9$) that had the

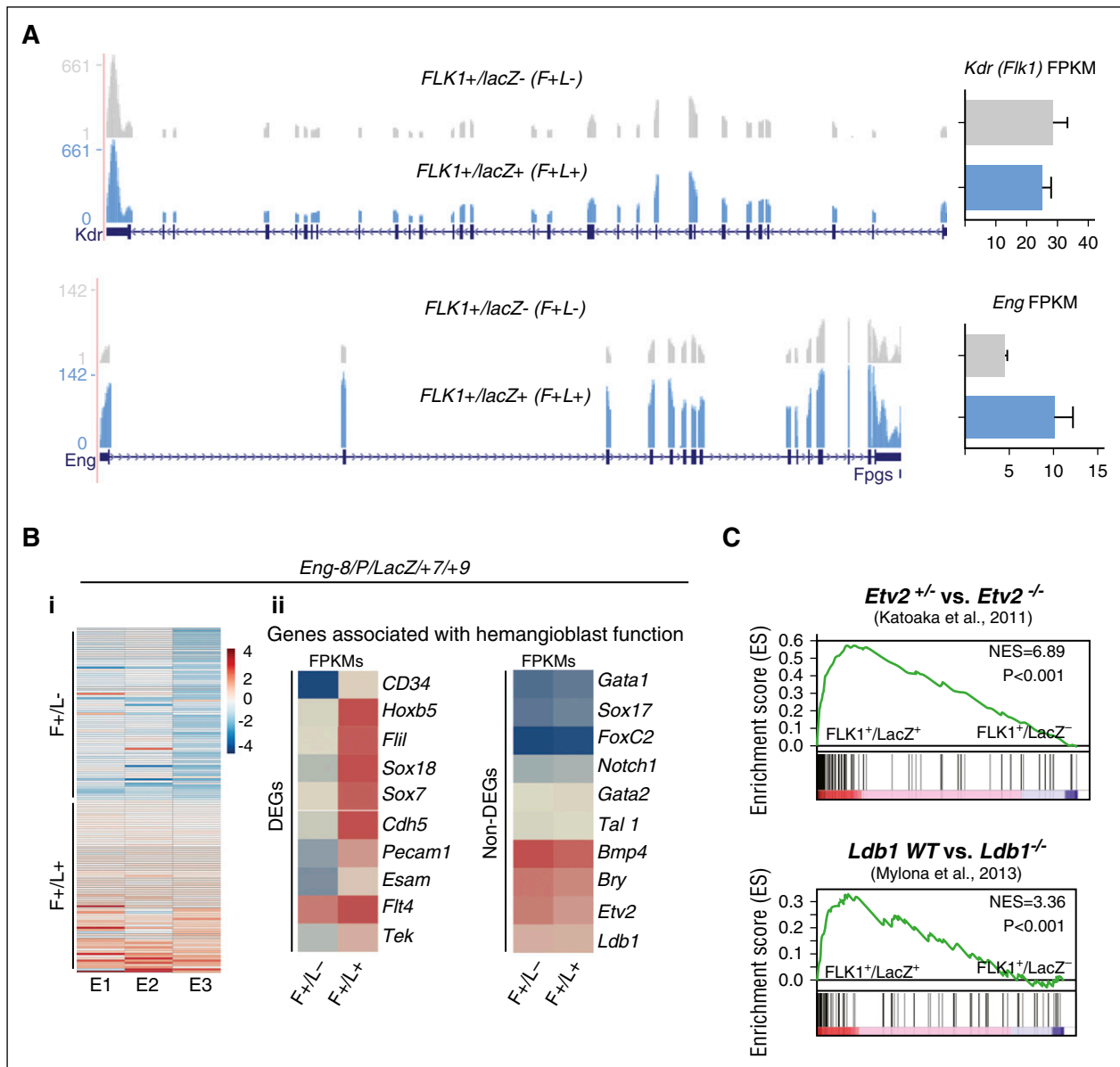


Figure 3. RNA sequencing of FLK1 mesoderm targeted by *Eng-8/P/LacZ+/7/+9* identifies genes associated with hemangioblast activity. (A) RNA-sequencing profiles shows *Kdr (Flk1)* transcripts (top) and *Eng* transcripts (bottom) in the F+/L- and F+/L+ fractions. FPKM expression values are shown to the right. (B) (i) Heat map representation of up- and downregulated genes in FLK1+/LacZ- (F+/L-) and FLK1+/LacZ+ (F+/L+) fractions in 3 independent experiments. (ii) Expression (FPKM values) levels of genes that have previously been associated with hemangioblast function. The left panel shows a subset of genes that are differentially expressed between F+/L- and F+/L+ fractions, and the right panel shows a subset of genes that are not. (C) GSEA profiles shows the correspondence of genes that are differentially expressed between F+/L- and F+/L+ fractions and those that are differentially expressed in *ETV2*^{+/-} vs *ETV2*^{-/-} (top) and *LDB* wt vs *LDB*^{-/-} gene sets. DEG, differentially expressed genes.

strongest and most specific activity in blood and endothelium in the developing embryo.¹⁷

Global transcriptomic analysis of FLK1 mesoderm targeted by *Eng-8/P/LacZ+/7/+9* identifies genes associated with hemangioblast activity

To discover genes associated with the activation of these GREs and increased activity of the reporters, we performed RNA sequencing on sorted *lacZ+* and *lacZ-* cell fractions from 3 independent experiments. As expected, *Kdr (Flk1)* expression was comparable in both fractions and *Eng* transcripts were increased in the *lacZ+* fraction consistent with *Eng* GRE-driven reporter activity (Figure 3A). There was also a shared set of genes that was consistently differentially expressed between

the *lacZ+* and *lacZ-* cell fractions (Figure 3Bi) and included 107 upregulated and 101 downregulated genes. These genes included cell surface receptors (CSRs) and TFs known to be associated with blood and endothelium (fold change ≥ 2 and *P* value $< .5$; supplemental Table 1; supplemental Figure 4A) and genes that have been associated with hemangioblast development in the LifeMap Sciences embryonic development compendium (Figure 3Bii). Individually, the expression of many genes known to be associated with early mesoderm (eg, *Bry/T* and *Bmp4*), blood (eg, *Gata1* and *Tal1*), and endothelial (eg, *Foxc2* and *Etv2*) development did not vary significantly between these cell fractions (Figure 3Bii). Indeed, as hemangioblasts are a subpopulation of FLK1+ mesoderm with multilineage differentiation potential, it would have been unusual to see significant differences in expression of individual genes that are strongly associated with commitment to a

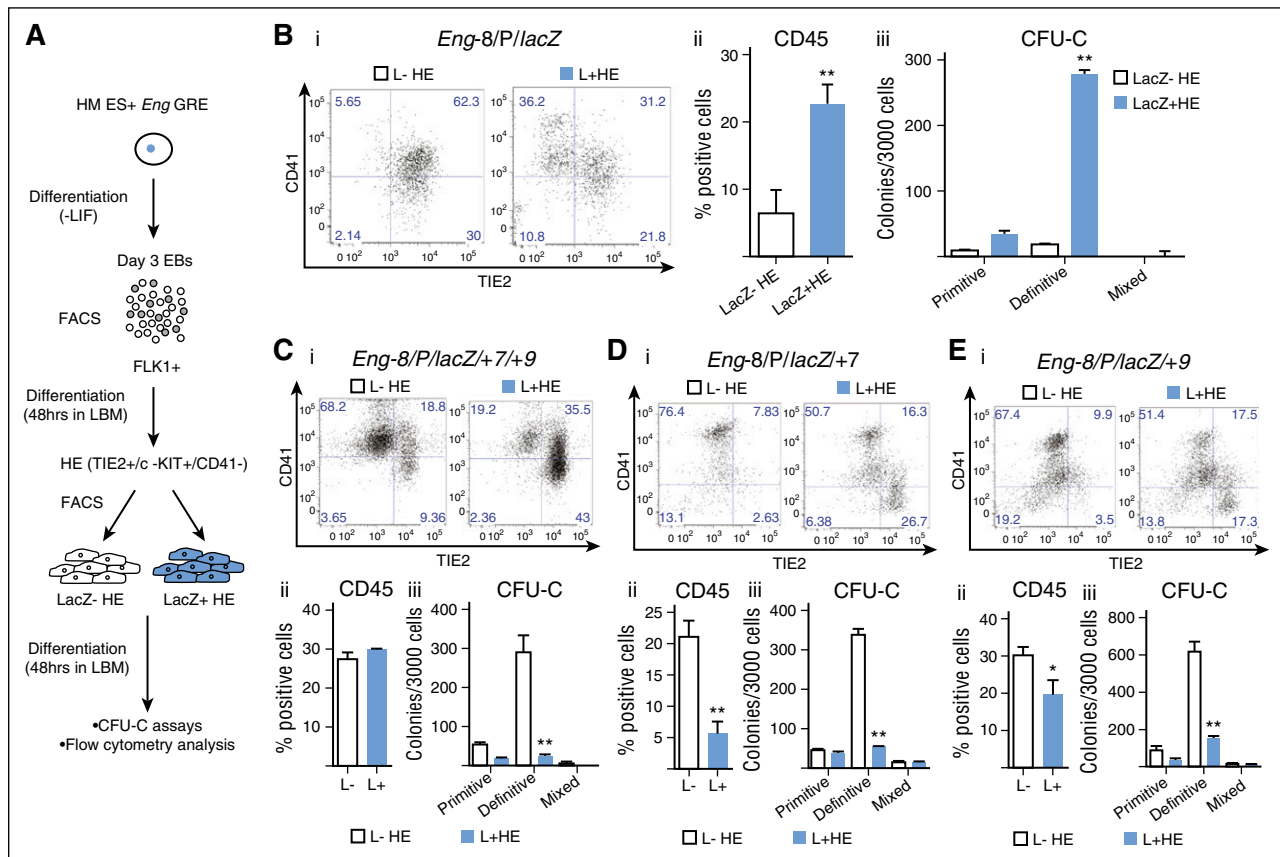


Figure 4. The *Eng* promoter in combination with the -8 endothelial enhancer targets HECs enriched for hematopoietic potential. (A) Schematic diagram outlining the experimental procedure. Recombinant ES cells generated using the *Eng* reporter constructs were differentiated into day 3 EBs. FLK1⁺ mesodermal cells were sorted from representative clones for each recombinant ES cell line and cultured in LBM. At 48 hours, CD41⁺/TIE2⁺/c-KIT⁺ HE cells were sorted into *lacZ*⁺ and *lacZ*⁻ fractions. The sorted fractions were recultured in LBM for a further 48 hours followed by flow cytometry and CFU-C assays. (B) (i) CD41 and TIE2 expression in sorted c-KIT⁺ HE *lacZ*⁻ (white) and *lacZ*⁺ (blue) fractions (after 2 days of reculture) derived from *Eng-8/P/lacZ* ES cells. (ii) Percentage of CD45⁺ cells generated from *lacZ*⁻ and *lacZ*⁺ HE fractions. (iii) Bar chart shows the number and type of hematopoietic colonies generated by each fraction. (C) (i-iii) Corresponding data to (B) generated from *Eng-8/P/lacZ/+7/+9* ES cells. (D) (i-iii) Corresponding data to (B) generated from *Eng-8/P/lacZ/+7* ES cells. (E) (i-iii) Corresponding data to (B) generated from *Eng-8/P/lacZ/+9* ES cells. Primitive and definitive colonies were scored after 4 and 9 days, respectively. Statistical analysis was conducted using Student *t* test, **P* < .05, ***P* < .01.

specific lineage. However, ingenuity pathway analysis (IPA) revealed that when differentially expressed genes were considered as a collective, there were strong associations with blood and blood vessel development for genes in the FLK1⁺/*lacZ*⁺ set (supplemental Figure 4B). Consistent with these biological functions, this gene set also showed significant associations with signaling pathways that govern endothelial development and endothelial nitric oxide synthase signaling (supplemental Figure 4C-F).

To investigate the *in vivo* relevance of our gene set, we used GSEA analysis to compare expression overlaps with gene expression data from FLK1⁺ cells in ETV2^{24,25} and LDB1²⁶ knockout embryos, both of which are defective in hemangioblasts (Figure 3C). There were strong overlaps between genes expressed in FLK1⁺ mesoderm targeted by *ENG-8/P/lacZ/+7/+9* and genes expressed in hemangioblast competent wild-type ETV2 and LDB1 embryos compared with ETV2^{+/-} or LDB1^{-/-} embryos, respectively. Therefore, the molecular signature of BL-CFC-enriched FLK1⁺ mesoderm that was identified using differential reporter activity of *ENG* GREs is consistent with *in vivo* functional capacity.

The *Eng* promoter in combination with the -8 endothelial enhancer targets HE cells enriched for hematopoietic potential

Definitive hematopoiesis in the embryo progresses through a TIE2⁺/c-KIT⁺/CD41⁻ HE intermediate.⁹ We used a cell culture system that mirrors this *in vivo* transition to investigate whether any of the reporter

ESC lines preferentially targeted HE cells and whether they could be used to isolate cell fractions that were enriched for hematopoietic potential.⁹ To this end, FLK1⁺ cells were sorted from day 3 EBs and seeded into liquid blast culture media (Figure 4A). At day 2 of culture, *lacZ*⁺ and *lacZ*⁻ HE cells were isolated by fluorescence-activated cell sorter and reseeded into liquid blast media (LBM) for 2 further days followed by flow cytometry and CFU-C assays to evaluate the hematopoietic potential of each fraction. Of the reporter ESC lines, *Eng-8/P/lacZ* was unique in that it was active in a fraction of HE cells that generated more TIE2⁻/CD41⁺ and CD45⁺ cells after 48 hours in culture (Figure 4Bi,ii) and contained almost all CFU-C potential (Figure 4Bii). Whereas *Eng-8/P/lacZ/+7/+9* targeted FLK1⁺ mesoderm with increased BL-CFC potential (Figure 2), it did not target HE cells with increased hematopoietic potential (Figure 4Ci-iii). Indeed, this and each of the other constructs targeted HE cells that had lower hematopoietic potential (Figure 4D,E; supplemental Figure 5). Taken together, these data showed that not only was there a specific combination of *ENG* GREs that targeted HE cells but also the combination was distinct from that which targeted BL-CFCs in FLK1⁺ mesoderm.

Hematopoietic potential is highest in *Eng-8/P/lacZ* targeted HE cells that do not as yet express surface *ENG*

Cell fate transitions are dynamic, and our purpose was to use these reporter constructs to capture HE cells that were intrinsically fated

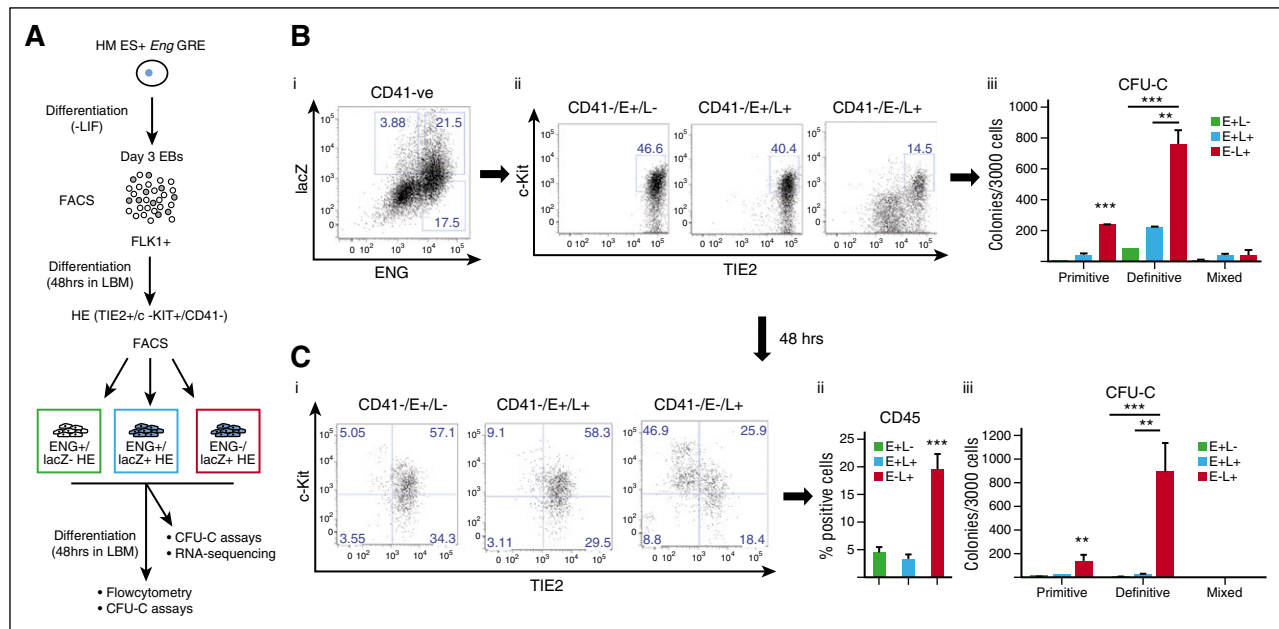


Figure 5. Hematopoietic potential is highest in *Eng*^{-8/P/lacZ} targeted HE cells that do not express surface ENG. (A) Schematic diagram outlining the experimental procedure. FLK1+ mesodermal cells were sorted from day 3 EBs generated from the *Eng*^{-8/P/lacZ} recombinant ES cell line and cultured in liquid blast culture media. At 48 hours, CD41⁻/TIE2⁺/c-KIT⁺ (HE) cells were sorted into ENG+/lacZ⁻, ENG+/lacZ⁺, and ENG-/lacZ⁺ fractions. These fractions were either directly seeded into CFU-C assays (B) or recultured in LBM for a further 48 hours and analyzed by flow cytometry and CFU-C assays (C). (B) (i-ii) Flow cytometry shows the frequencies of CD41⁻/TIE2⁺/c-KIT⁺ (HE) cells in ENG+/lacZ⁺, ENG+/lacZ⁻, and ENG-/lacZ⁺ fractions. (iii) CFU-C potential of each sorted fraction in (i). (C) (i) Flow cytometry analysis of CD41 and TIE2 expression in sorted HE cell fractions after 2 days of reculture in LBM. (ii) Bar chart shows the percentage of CD45⁺ positive cells in sorted fraction. (iii) Bar chart shows hematopoietic colony numbers from each fraction. Primitive and definitive colonies were scored after 4 and 9 days, respectively. Statistical analysis was conducted using Student *t* test, ***P* < .01, and ****P* < .001.

toward the hematopoietic lineage at the earliest possible time point in culture. Based on the assumption that there would be a delay between transactivating the *Eng* GRE reporter and surface expression of ENG, we repeated the experiments described in Figure 4 using the *Eng*^{-8/P/lacZ} ES cell line but here also incorporating surface ENG expression to isolate TIE2⁺/c-KIT⁺/CD41⁻ HE fractions that were ENG+/lacZ⁻, ENG+/lacZ⁺, or ENG-/lacZ⁺ (Figure 5A). Interestingly, CFU-C potential within the lacZ⁺ fraction was highest in ENG-/lacZ⁺ HE cells (Figure 5Bi,ii). ENG+/lacZ⁻, ENG+/lacZ⁺, and ENG-/lacZ⁺ HE cells were reseeded in LBM and analyzed by flow cytometry and CFU-C assays after 2 further days of culture. The proportions of TIE2⁻/CD41⁺ and CD45⁺ (Figure 5Ci) cells and CFU-C potential (Figure 5Cii) were highest for cultured ENG-/lacZ⁺ HE cells. Taken together, these data show that the hematopoietic potential within HE cells can be targeted by *Eng*^{-8/P/lacZ} and that these ESCs could be used to interrogate the earliest transcriptional changes associated with this cell fate decision.

Transcriptomic analysis of HE fractions identifies genes associated with HE to hematopoietic transition

To discover genes that act on *Eng*^{-8/P/lacZ} and drive reporter gene activity and by extension are associated with hemogenic potential in TIE2⁺/c-KIT⁺/CD41⁻ HE cells, we performed RNA sequencing on sorted ENG+/lacZ⁻, ENG+/lacZ⁺, and ENG-/lacZ⁺ HE cell fractions from 3 independent experiments. As expected, the fractions, which expressed surface ENG, had abundant *Eng* transcripts, which were still comparatively low in ENG-/lacZ⁺ HE cells (Figure 6A). Consistent with its role as a major determinant of endothelial-to-hematopoietic transition (EHT),^{9,27,28} *Runx1* transcripts were abundant in HE cells that were enriched with functional hemogenic cells (ENG-/lacZ⁺ HE) and relatively low in those (ENG+/lacZ⁻ and ENG+/lacZ⁺ HE) that were not. In total, there were 707 upregulated and 981

downregulated genes in ENG-/lacZ⁺ HE cells compared with ENG+/lacZ⁻ and ENG+/lacZ⁺ HE cells (Figure 6Bi; supplemental Table 2). It was interesting to note that only a subset of genes that have previously been attributed to mark HE cells based on cell surface protein expression were¹⁶ differentially expressed between these functionally distinct HE subpopulations (Figure 6Bii). This observation does not imply that these genes are not important but that their higher or lower expression is not associated with these early subtle transitions.

To investigate the in vivo relevance of our gene set, we used GSEA analysis to compare expression overlaps between ENG-/lacZ⁺ HE vs ENG+/lacZ⁻ HE and gene sets generated from primary embryonic endothelial cell (EC), hemogenic endothelial cells (HECs), and HSCs.¹⁶ Consistent with our functional data, the gene sets associated with EC to HE transition (Figure 6Ci) and HE to HSC transition (Figure 6Cii) showed strong overlaps with genes expressed in ENG-/lacZ⁺ HE. Gene sets associated with HIF1a and DNA replication also showed strong overlaps with genes expressed in ENG-/lacZ⁺ HE cells (supplemental Figure 6A). Genes that were UP in ENG-/lacZ⁺ HE compared with ENG+/lacZ⁻ HE cells feature prominently in IPA reconstructions of gene networks governing hematopoietic development (supplemental Figure 6B). Whereas genes that were UP in ENG-/lacZ⁺ HE cells compared with either ENG+/lacZ⁻ HE or ENG+/lacZ⁺ HE cells were associated with biological processes relating to blood development, genes that were DOWN in ENG-/lacZ⁺ HE cells relative to the other 2 fractions were associated more with angiogenesis or vasculogenesis (supplemental Figure 6C-E). Interrogation of differentially expressed TFs and CSRs in the more functionally hemogenic ENG-/lacZ⁺ HE fraction relative to the ENG+/lacZ⁻ HE fraction showed upregulation of a number of TFs (eg, *Runx1*,²⁹ *Myb*,³⁰ *Gfi1b*,³¹) and CSRs (*Lgr5*³²) that are known to play a role in HSC development and downregulation of others (eg, *Sox17*³³), which are important for HE to HSC transition (Figure 6D).

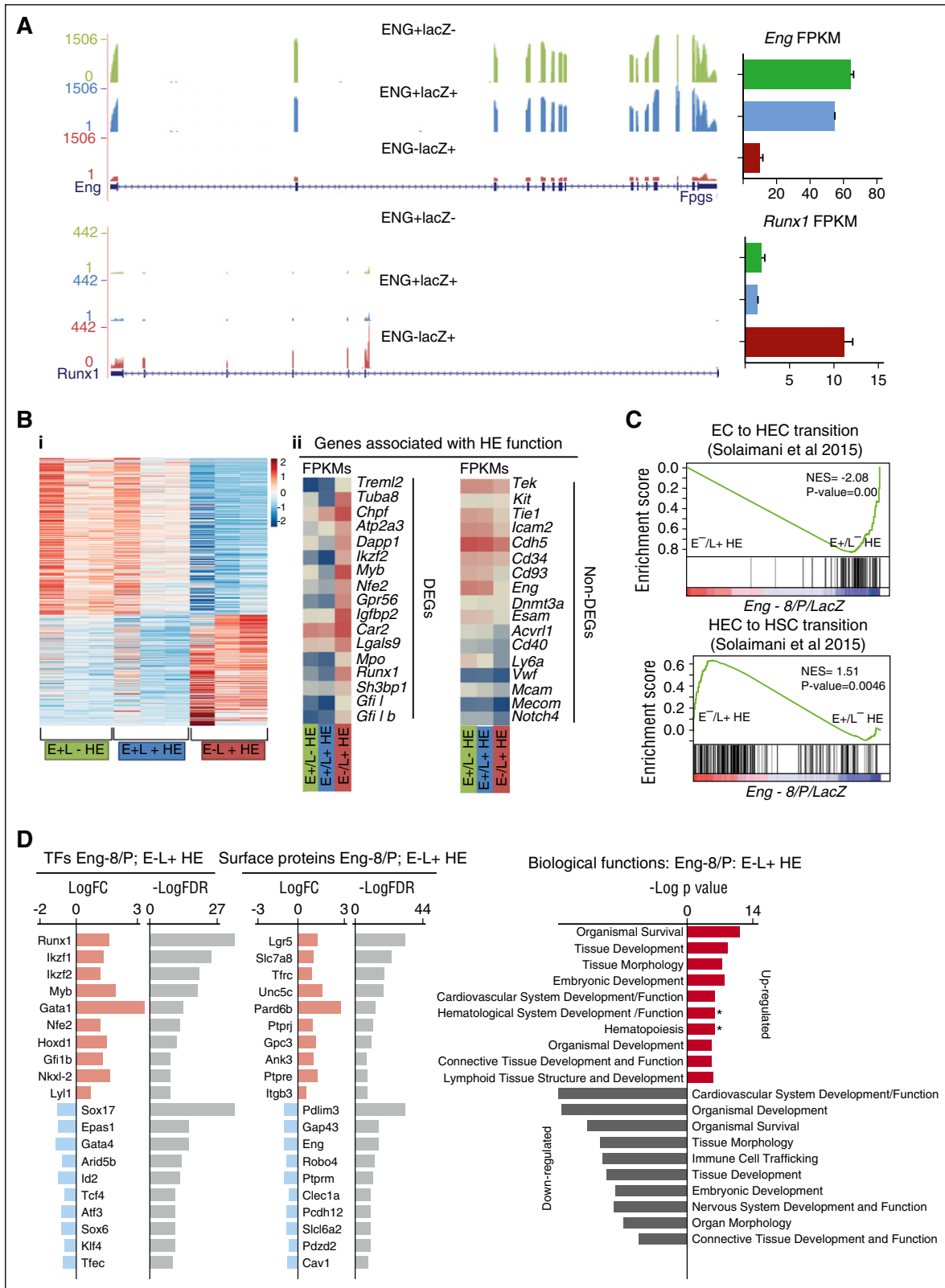


Figure 6. Transcriptomic analysis of HE fractions identifies genes associated with HE to hematopoietic transition. (A) RNA-sequencing profiles show *Eng* transcripts (top) and *Runx1* transcripts (bottom) in the E+/L-, E+/L+, and E-/L+ fractions. FPKM expression values are shown to the right. (B) (i) Heat map representation of up- and downregulated genes in ENG+/LacZ- (E+/L-) HE, ENG+/LacZ+ (E+/L+) HE, and ENG-/LacZ+ (E-/L+) HE fractions in 3 independent experiments. (ii) Expression (FPKM values) levels of genes that have previously been associated with HE. The top panel shows a subset of genes that are differentially expressed between E+/L-, E+/L+, and E-/L+ fractions, and the bottom panel shows a subset of genes that are not. (C) GSEA profiles show the correspondence of genes that are differentially expressed between the E+/L-, E+/L+, and E-/L+ fractions and those that are differentially expressed in EC vs HECs (top) and HECs vs HSC gene sets. (D) TFs and CSRs that are up- and downregulated in the *Eng* -8/P E-/L+ HE fraction. The log fold changes (logFC) and log false discovery rates (logFDR) are listed for each gene.

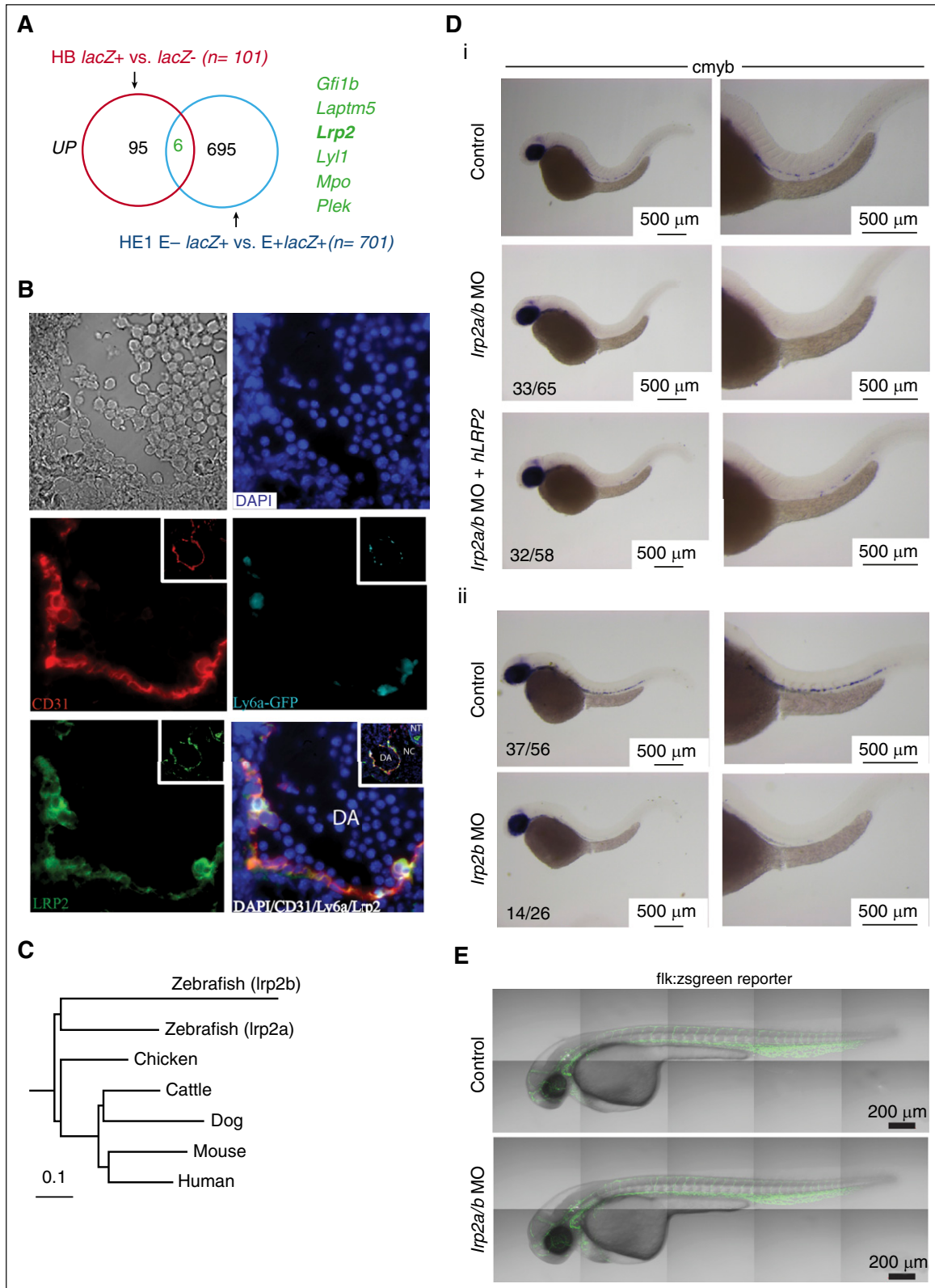


Figure 7. *Lrp2* is required for normal definitive hematopoiesis. (A) Venn diagram shows the overlap of genes that are UP in FLK1 mesoderm enriched for BL-CFCs and/or HE cells enriched for hemogenic potential. (B) Immunohistochemistry of E10.5 *Ly6aGFP* AGM shows coexpression of GFP and LRP2 in ECs and hematopoietic clusters. The insets show the same sections at low magnification. (C) Homology relationships of zebrafish *Irp2a* and *Irp2b* coding sequences with that of *Lrp2* in different vertebrate species. (D) ISH for the HSC marker *cmyb* in zebrafish at 36 hpf. (i) Low (left) and high (right) magnification images of control zebrafish (top row), *Irp2a/b* morpholinos (middle row), and *Irp2a/b* morpholinos coinjected with *hLRP2* mRNA (bottom row) zebrafish. (ii) Low (left) and high (right) magnification images of control zebrafish (top row) and *Irp2b* morpholinos. (E) Confocal images of *flk:zsgreen* reporter embryos show an intact vasculature in both control (top) and *Irp2a/b* morphant (bottom) embryos. DA, dorsal aorta; NC, notochord; NT, neural tube.

Lrp2 is required for normal blood emergence in the zebrafish aorta

We then overlapped our gene sets to visualize associations between genes that were UP or DOWN in HB and/or HE (supplemental Figures 7 and 8; supplemental Tables 1 and 2) to interrogate their function. Six genes were shared between the upregulated groups (Figure 7A; supplemental Figure 7C) and 8 genes between the downregulated groups (supplemental Figure 8A). Genes that were DOWN in both HB and HE cells included several with no known association with hematopoiesis (supplemental Figure 8B). However, we focused on genes that were UP in both HB and HE cells (Figure 7A; supplemental Figure 7C) for practical considerations given that their expression and functional role would be easier to validate. This group included hematopoietic TFs (*Gfi1*³¹ and *Lyl1*³⁴), a platelet protein kinase C substrate (*Plek*³⁵) and granulocyte lysosomal and lysosomal membrane proteins (*Mpo*³⁶ and *Laptm5*³⁷), all of which have known functions in the hematopoietic system. It also included a multifunctional ligand (*Lrp2*) with no previously described role in blood or blood development. *Lrp2*/Megalin is a member of an endocytic receptor complex that is involved in maternal-fetal transport of folate and other nutrients, lipids, and morphogens such as sonic hedgehog (Shh) and retinoids.³⁸ Given these associations, we postulated that *Lrp2* upregulation in blood precursors was likely to be of functional significance.

The *Ly6aGFP* (Sca1) mouse model, in which all HSCs throughout development are GFP+,^{14,39} has facilitated the study of EHT. These mice were used to show in real time the transition of morphologically flat endothelial GFP+ cells in the E10.5 aorta to round GFP+ cells that coexpress other HSC markers.⁴⁰ Given that LRP2 was upregulated in HE cells, we evaluated LRP2 expression in *Ly6aGFP* E10.5 AGM. LRP2 shows specific expression in ECs with strong expression in *Ly6aGFP*+ ECs and hematopoietic clusters (Figure 7B).

EHT is an evolutionarily conserved process in vertebrates, and real-time imaging of transgenic zebrafish embryos has also shown the transition of aortic ECs to hematopoietic cells.^{41,42} *Lrp2* is highly conserved across different vertebrate species (Figure 7C; average sequence identity across all species shown = 70%). The zebrafish genome has 2 closely related protein-coding genes, *lrp2a* on chromosome 9 and *lrp2b* on chromosome 12, both of which are expressed at 24 to 72 hpf.⁴³ To validate the involvement of LRP2 in HSC generation, we used a zebrafish morpholino oligo (MO) knockdown approach targeting both *lrp2a* and *2b* together and each alone. At 36 hours postfertilization (hpf), morphants were assayed by ISH for *cmyb* and *runx1*, markers for emerging blood progenitors in the aorta.⁴⁴ Wild-type embryos showed robust *cmyb* and *runx1* expressing cells along the dorsal aorta in contrast to *lrp2a/b* morphants that showed severe reductions (Figure 7Di; supplemental Figure 9A). There was partial rescue of AGM blood progenitors when *lrp2a/b* morphants were coinjected with *hLRP2* mRNA. The partial rescue was probably due to only partial homology of protein sequences between humans and fish (~65%) and quality of in vitro transcribed mRNA given the large size of *LRP2* cDNA (~14 kb). To exclude nonspecific toxicity-related loss of *cmyb* and *runx1* expressing cells, we coinjected *lrp2a/b* MO with *tp53* MO and saw no restitution of *cmyb* expressing cells in the morphants (supplemental Figure 9B). Injection of *lrp2b*MO, but not *lrp2a*MO, reduced the numbers of *cmyb* expressing AGM blood progenitors (*lrp2b*MO; Figure 7Dii; *lrp2a*; data not shown). To establish that this defect in blood cell production was not secondary to loss of vascular integrity, we injected *lrp2a/b* MO into flk:zsgreen transgenic embryos and saw no difference between morphants and

controls at 32 hpf (Figure 7E). In addition to vascular integrity, we also assessed blood flow in morphants. Both heart function and blood flow was indistinguishable from control embryos (data not shown). Taken together, these data support a role for LRP2 during AGM hematopoiesis.

Discussion

Regulatory elements of genes that demonstrate tissue-specific expression have previously been used to target and characterize various cell populations in ESC systems.⁴⁵⁻⁴⁸ When initiating these experiments, we did not envisage that distinct combinations of ENG promoter/enhancers would target hemangioblast potential in FLK1 mesoderm and hemogenic potential in TIE2+/C-KIT+/CD41- hemogenic endothelium. In retrospect, given the distinct transcriptomes and functional properties of hemangioblasts and hemogenic endothelium, this should not have come as a surprise, nor did we predict that hemogenic potential would be enriched in ENG- HE1 cells targeted by the *ENG-8PlacZ* transgene. Given that F+L+ cells expressed higher levels of ENG (Figure 3A), these data raise the question whether HE1 cells emerge from F+L- ENG low cells, which are less able than their F+L+ counterparts to generate BL-CFCs or whether F+L+ ENG high cells subsequently shutdown ENG expression to facilitate their hemogenic potential in HE1 cells. As these populations were targeted by different transgenes (F+L+; *Eng-8P/lacZ*+7/+9 and ENG-L+ HE; *Eng-8P/lacZ*), this could not be directly tested. However, ES cells targeted with dual reporters each driven by either *Eng-8P*+7/+9 or *Eng-8P* may assist in addressing this specific question.

Lrp2, a gene that encodes megalin, a multiligand uptake receptor that regulates circulating levels of diverse compounds,⁴⁹ emerged as a novel regulator of hematopoiesis. Mutations in LRP2 result in impaired neuroepithelial development and are causative of Donnai-Barrow and facio-oculo-acoustico-renal syndromes.⁵⁰ It has been implicated in balancing BMP4 and SHH signaling in neuroepithelium by acting as a clearance receptor for BMP4 and by concentrating or depleting SHH by ligand recycling or clearance, respectively, in a cell-type and context-dependent manner.⁵¹ This control mechanism is of interest as the BMP4-SHH gradient between the neural tube and dorsal aorta has also been implicated in the induction of the HSC developmental program in the ventral wall of the dorsal aorta.⁵² On a C57Bl/6N background, the LRP2 mutation causes lethality in mice around the time of birth, and there are no mutant pups, although embryo collections at all embryonic stages to E18.5 show expected Mendelian ratios. LRP2 mutations on 129 or CD1 backgrounds also do not yield survivors (A.H. and T.W., unpublished data). On a FVB/N background, however, LRP2 null mice are viable with neural tube defects, and this receptor has previously been implicated in folate endocytosis in the developing neural tube.⁵³ However, peripheral blood and bone marrow hematopoietic stem and progenitor cell numbers were comparable in FVB/N wild-type and mutant adult mice at 6 to 9 months of age (supplemental Figure 10). A more detailed analysis of embryonic hematopoiesis in mutant mice on both C57Bl/6N and FVB/N backgrounds will be required to establish whether the numbers of emergent HSCs differ at various time points and the identity of any modifier genes in FVB/N that compensate for the loss of LRP2; these investigations are ongoing. However, taken together with the zebrafish data, which show reduction rather than loss of HSCs, LRP2 is likely to facilitate rather than be absolutely required for EHT. Indeed, it is important to keep in mind that *Lrp2* transcripts were higher in HB cells with

greater BL-CFC potential and HE cells with greater CFU-C potential, but cells with lower numbers of transcripts were also able to generate BL-CFCs and CFU-Cs.

Deficiency of dietary folate also results in impaired neural tube development and megaloblastic anemia.⁵⁴ Targeted inactivation of the reduced folate carrier, which facilitates folate delivery into cells, results in embryonic lethality at E10.5 due to neural and hematopoietic defects,⁵⁵ and components of the Megalin complex are among the most significantly disrupted genes in null embryos.³⁸ Coordinated upregulation of a receptor that facilitates folate uptake in HECs would be consistent with demand for an essential hematonic in cells that are on the threshold of a replicative phase.

Although we focused our attention on *Lrp2*, as a gene without a described role in hematopoiesis, from a list of 6 that were upregulated in both hemangioblasts and HE cells, there were other genes that were UP in only one or the other cell fraction. Given the overlap of these gene sets with those generated from gene knockout embryos with specific developmental defects, they will serve as a rich resource to explore and manipulate the emergence of hemangioblasts from FLK1+ mesoderm or hematopoiesis in HE cells. Insights gained from these manipulations will in turn inform tissue regeneration protocols that aim to generate functional HSCs.

Acknowledgments

This work was supported by grants from the National Health and Medical Research Council of Australia, Australian Research Council, and the Tom Bee Stem Cell Research Fund (J.E.P.), Cancer Research UK (V.K. and G.L.), the Biotechnology and Biological Sciences Research Council, Leukaemia and Lymphoma Research, The Leukaemia and Lymphoma Society, and core support grants by the Wellcome Trust to the Cambridge Institute for Medical Research and Wellcome Trust–Medical Research Council Cambridge Stem

Cell Institute (B.G.). This work was supported by grants from the National Institutes of Health National Heart, Lung, and Blood Institute (R01 HL04880, P015PO1HL32262-32, and 5U01 HL10001-05) and National Institute of Diabetes and Digestive and Kidney Diseases (5P30 DK49216, 5R01 DK53298, and R24 DK092760) (L.I.Z.). E.M.F. is a Fellow of the Leukemia and Lymphoma Society.

Authorship

Contribution: R.N., E.M.F., P.S., K.K., R.T., M.S., F.P., J.K., A.U., J.T., D.B., C.S.V., A.E., and R.P. performed research and analyzed data. A.S., J.W., A.H., D.K., R.R., T.W., B.G., E.D., and L.I.Z. contributed essential reagents and advice with data analysis and interpretation. R.N., G.L., V.K., and J.E.P. contributed to study design, data interpretation, and manuscript preparation. All authors read and approve the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

The current affiliation for P.S. is BeDataDriven BV, The Hague, The Netherlands.

The current affiliation for A.S. is GSK Pharmaceutical Company, London, United Kingdom.

Correspondence: John E. Pimanda, Lowy Cancer Research Centre and the Prince of Wales Clinical School, UNSW Australia, Sydney, NSW 2052, Australia; e-mail: jpimanda@unsw.edu.au; Valerie Kouskoff, Cancer Research United Kingdom Manchester Institute, The University of Manchester, 553 B5093, Manchester M20 4BX, United Kingdom; e-mail: valerie.kouskoff@cruk.manchester.ac.uk; Georges Lacaud, Cancer Research United Kingdom Manchester Institute, The University of Manchester, 553 B5093, Manchester M20 4BX, United Kingdom; e-mail: georges.lacaud@cruk.manchester.ac.uk.

References

- Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005;121(7):1109-1121.
- Notta F, Doulatov S, Laurenti E, Poeschl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science*. 2011;333(6039):218-221.
- Riddell J, Gazit R, Garrison BS, et al. Reprogramming committed murine blood cells to induced hematopoietic stem cells with defined factors [published correction appears in *Cell*. 2014 Jul 3;158(1):226]. *Cell*. 2014;157(3):549-564.
- Pereira CF, Lemischka IR, Moore K. "From blood to blood": de-differentiation of hematopoietic progenitors to stem cells. *EMBO J*. 2014;33(14):1511-1513.
- Medvinsky A, Rytsov S, Taoudi S. Embryonic origin of the adult hematopoietic system: advances and questions. *Development*. 2011;138(6):1017-1031.
- Huber TL, Kouskoff V, Fehling HJ, Palis J, Keller G. Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. *Nature*. 2004;432(7017):625-630.
- Fehling HJ, Lacaud G, Kubo A, et al. Tracking mesoderm induction and its specification to the hemangioblast during embryonic stem cell differentiation. *Development*. 2003;130(17):4217-4227.
- Rytsov S, Sobiesiak M, Taoudi S, et al. Hierarchical organization and early hematopoietic specification of the developing HSC lineage in the AGM region. *J Exp Med*. 2011;208(6):1305-1315.
- Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V, Lacaud G. The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. *Nature*. 2009;457(7231):892-895.
- Nasrallah R, Knezevic K, Thai T, et al. Endoglin potentiates nitric oxide synthesis to enhance definitive hematopoiesis. *Biol Open*. 2015;4(7):819-829.
- Davidson EH. The regulatory genome: gene regulatory networks in development and evolution. Amsterdam, London: Elsevier/Academic Press; 2006.
- Beck D, Thoms JA, Perera D, et al. Genome-wide analysis of transcriptional regulators in human HSPCs reveals a densely interconnected network of coding and noncoding genes. *Blood*. 2013;122(14):e12-e22.
- Swiers G, Baumann C, O'Rourke J, et al. Early dynamic fate changes in haemogenic endothelium characterized at the single-cell level. *Nat Commun*. 2013;4:2924.
- Ma X, Robin C, Ottersbach K, Dzierzak E. The Ly-6A (Sca-1) GFP transgene is expressed in all adult mouse hematopoietic stem cells. *Stem Cells*. 2002;20(6):514-521.
- Chen CZ, Li M, de Graaf D, et al. Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. *Proc Natl Acad Sci USA*. 2002;99(24):15468-15473.
- Solaimani Kartalaei P, Yamada-Inagawa T, Vink CS, et al. Whole-transcriptome analysis of endothelial to hematopoietic stem cell transition reveals a requirement for Gpr56 in HSC generation. *J Exp Med*. 2015;212(1):93-106.
- Pimanda JE, Chan WY, Wilson NK, et al. Endoglin expression in blood and endothelium is differentially regulated by modular assembly of the Ets/Gata hemangioblast code. *Blood*. 2008;112(12):4512-4522.
- Pimanda JE, Göttgens B. Gene regulatory networks governing haematopoietic stem cell development and identity. *Int J Dev Biol*. 2010;54(6-7):1201-1211.
- Cheifetz S, Bellón T, Calés C, et al. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. *J Biol Chem*. 1992;267(27):19027-19030.
- Perlingeiro RC. Endoglin is required for hemangioblast and early hematopoietic development. *Development*. 2007;134(16):3041-3048.

21. Li DY, Sorensen LK, Brooke BS, et al. Defective angiogenesis in mice lacking endoglin. *Science*. 1999;284(5419):1534-1537.
22. Pimanda JE, Chan WY, Donaldson IJ, Bowen M, Green AR, Göttgens B. Endoglin expression in the endothelium is regulated by Fli-1, Erg, and Elf-1 acting on the promoter and a -8-kb enhancer. *Blood*. 2006;107(12):4737-4745.
23. Magin TM, McWhir J, Melton DW. A new mouse embryonic stem cell line with good germ line contribution and gene targeting frequency. *Nucleic Acids Res*. 1992;20(14):3795-3796.
24. Wareing S, Mazan A, Pearson S, Göttgens B, Lacaud G, Kouskoff V. The Flk1-Cre-mediated deletion of ETV2 defines its narrow temporal requirement during embryonic hematopoietic development. *Stem Cells*. 2012;30(7):1521-1531.
25. Kataoka H, Hayashi M, Nakagawa R, et al. Etv2/ER71 induces vascular mesoderm from Flk1+ PDGFR α + primitive mesoderm. *Blood*. 2011;118(26):6975-6986.
26. Mylona A, Andrieu-Soler C, Thongjuea S, et al. Genome-wide analysis shows that Ldb1 controls essential hematopoietic genes/pathways in mouse early development and reveals novel players in hematopoiesis. *Blood*. 2013;121(15):2902-2913.
27. Sroczyńska P, Lancrin C, Kouskoff V, Lacaud G. The differential activities of Runx1 promoters define milestones during embryonic hematopoiesis. *Blood*. 2009;114(26):5279-5289.
28. Chen MJ, Yokomizo T, Zeigler BM, Dzierzak E, Speck NA. Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature*. 2009;457(7231):887-891.
29. Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell*. 1996;84(2):321-330.
30. Mucenski ML, McLain K, Kier AB, et al. A functional c-myc gene is required for normal murine fetal hepatic hematopoiesis. *Cell*. 1991;65(4):677-689.
31. Lancrin C, Mazan M, Stefanska M, et al. GF11 and GF11B control the loss of endothelial identity of hemogenic endothelium during hematopoietic commitment. *Blood*. 2012;120(2):314-322.
32. Liu D, He XC, Qian P, et al. Leucine-rich repeat-containing G-protein-coupled Receptor 5 marks short-term hematopoietic stem and progenitor cells during mouse embryonic development. *J Biol Chem*. 2014;289(34):23809-23816.
33. Lizama CO, Hawkins JS, Schmitt CE, et al. Repression of arterial genes in hemogenic endothelium is sufficient for haematopoietic fate acquisition. *Nat Commun*. 2015;6:7739.
34. Capron C, Lécluse Y, Kaushik AL, et al. The SCL relative LYL-1 is required for fetal and adult hematopoietic stem cell function and B-cell differentiation. *Blood*. 2006;107(12):4678-4686.
35. Wehrspaun CC, Haerty W, Ponting CP. Microglia recapitulate a hematopoietic master regulator network in the aging human frontal cortex. *Neurobiol Aging*. 2015;36(8):2443 e2449-2443 e2420.
36. Le Guyader D, Redd MJ, Colucci-Guyon E, et al. Origins and unconventional behavior of neutrophils in developing zebrafish. *Blood*. 2008;111(1):132-141.
37. Adra CN, Zhu S, Ko JL, et al. LAPTM5: a novel lysosomal-associated multispansing membrane protein preferentially expressed in hematopoietic cells. *Genomics*. 1996;35(2):328-337.
38. Gelineau-van Waes J, Maddox JR, Smith LM, et al. Microarray analysis of E9.5 reduced folate carrier (RFC1; Slc19a1) knockout embryos reveals altered expression of genes in the cubilin-megalin multiligand endocytic receptor complex. *BMC Genomics*. 2008;9:156.
39. de Bruijn MF, Ma X, Robin C, Ottersbach K, Sanchez MJ, Dzierzak E. Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. *Immunity*. 2002;16(5):673-683.
40. Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature*. 2010;464(7285):116-120.
41. Bertrand JY, Chi NC, Santoso B, Teng S, Stainier DY, Traver D. Haematopoietic stem cells derive directly from aortic endothelium during development. *Nature*. 2010;464(7285):108-111.
42. Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. *Nature*. 2010;464(7285):112-115.
43. Kur E, Christa A, Veth KN, et al. Loss of Lrp2 in zebrafish disrupts pronephric tubular clearance but not forebrain development. *Dev Dyn*. 2011;240(6):1567-1577.
44. Jing L, Zon LI. Zebrafish as a model for normal and malignant hematopoiesis. *Dis Model Mech*. 2011;4(4):433-438.
45. Ferreira R, Spensberger D, Silber Y, et al. Impaired in vitro erythropoiesis following deletion of the Scl (Tal1) +40 enhancer is largely compensated for in vivo despite a significant reduction in expression. *Mol Cell Biol*. 2013;33(6):1254-1266.
46. Wu SM, Fujiwara Y, Cibulsky SM, et al. Developmental origin of a bipotential myocardial and smooth muscle cell precursor in the mammalian heart. *Cell*. 2006;127(6):1137-1150.
47. Holtzinger A, Rosenfeld GE, Evans T. Gata4 directs development of cardiac-inducing endoderm from ES cells. *Dev Biol*. 2010;337(1):63-73.
48. Feng G, Mellor RH, Bernstein M, et al. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron*. 2000;28(1):41-51.
49. Saito A, Pietromonaco S, Loo AK, Faruqar MG. Complete cloning and sequencing of rat gp330/“megalin,” a distinctive member of the low density lipoprotein receptor gene family. *Proc Natl Acad Sci USA*. 1994;91(21):9725-9729.
50. Kantarci S, Al-Gazali L, Hill RS, et al. Mutations in LRP2, which encodes the multiligand receptor megalin, cause Donnai-Barrow and facio-oculo-acoustico-renal syndromes. *Nat Genet*. 2007;39(8):957-959.
51. Christ A, Christa A, Klippert J, et al. LRP2 acts as SHH clearance receptor to protect the retinal margin from mitogenic stimuli. *Dev Cell*. 2015;35(1):36-48.
52. Wilkinson RN, Pouget C, Gering M, et al. Hedgehog and Bmp polarize hematopoietic stem cell emergence in the zebrafish dorsal aorta. *Dev Cell*. 2009;16(6):909-916.
53. Kur E, Mecklenburg N, Cabrera RM, Willnow TE, Hammes A. LRP2 mediates folate uptake in the developing neural tube. *J Cell Sci*. 2014;127(Pt 10):2261-2268.
54. Bills ND, Koury MJ, Clifford AJ, Dessypris EN. Ineffective hematopoiesis in folate-deficient mice. *Blood*. 1992;79(9):2273-2280.
55. Gelineau-van Waes J, Heller S, Bauer LK, et al. Embryonic development in the reduced folate carrier knockout mouse is modulated by maternal folate supplementation. *Birth Defects Res A Clin Mol Teratol*. 2008;82(7):494-507.



blood[®]

2016 128: 1928-1939

doi:10.1182/blood-2016-02-697870 originally published
online August 23, 2016

Identification of novel regulators of developmental hematopoiesis using *Endoglin* regulatory elements as molecular probes

Rabab Nasrallah, Eva M. Fast, Parham Solaimani, Kathy Knezevic, Alexia Eliades, Rahima Patel, Roshana Thambyrajah, Ashwin Unnikrishnan, Julie Thoms, Dominik Beck, Chris S. Vink, Aileen Smith, Jason Wong, Mairi Shepherd, David Kent, Rahul Roychoudhuri, Fabian Paul, Julia Klippert, Annette Hammes, Thomas Willnow, Bertie Göttgens, Elaine Dzierzak, Leonard I. Zon, George Lacaud, Valerie Kouskoff and John E. Pimanda

Updated information and services can be found at:

<http://www.bloodjournal.org/content/128/15/1928.full.html>

Articles on similar topics can be found in the following Blood collections

[Hematopoiesis and Stem Cells](#) (3367 articles)

Information about reproducing this article in parts or in its entirety may be found online at:

http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:

<http://www.bloodjournal.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:

<http://www.bloodjournal.org/site/subscriptions/index.xhtml>

SUPPLEMENTAL METHODS

Murine ES cell culture

The Bry/GFP ¹ and HM1 ES cells ² have been previously described. Murine ES cells were seeded on 6-well plates coated with irradiated mouse embryonic feeders, in media composed of 84% Dulbecco's Modified Eagle Medium (DMEM, PAA Laboratories), 15% Fetal Bovine serum (FBS, Invitrogen) pre-tested for maintenance of ES cells, 0.1% of 0.15M dilution of monothioglycerol (MTG, Sigma Aldrich) and 1000units/ml mouse recombinant Leukaemia inhibitory factor (Esgro, Millipore).

Generating *Hprt* targeting constructs

To generate the *Hprt* targeting constructs DNA fragments corresponding to the *Eng* -8, *P*, +7, +9 ^{3,4}, *mutant* +7 (+7Δ, *mutant Gata*), and *mutant* +9 (+9Δ, *mutant Ets*) regions (Fig. S1B) along with the *LacZ* reporter gene were ligated in multiple combinations into the pMP8 targeting vector. The DNA constructs cloned into pMP8 were sequenced using the following primers; Pmp8_F, 5'-AGAACGTCAGTAGTCATAG-3'; Pmp8_R, 5'-TGCAGTGAGCCAGACTGTG-3'. The generated targeting constructs are listed in supplementary figure S1C. A map for *Eng* -8/*P* in pMP8 can be found in Supplementary Fig. S2A (All other reporter constructs were cloned into the same region of the pMP8 vector).

Hprt targeting of HM1 ES cells

To prepare the cells for targeting, HM1 ES cells were cultured in DMEM-ES medium containing 1× 6-Thioguanine (6-TG, Sigma Aldrich) for a week before electroporation. The cells were grown to confluence, washed with PBS, and trypsinized to obtain a single cell

suspension. 1×10^8 HM1 cells were resuspended in 900 μ l ice-cold PBS, mixed with 100 μ g of PmeI-linearized targeting vector and electroporated at 0.8Kv/3uF using a Gene Pulser (BioRad). Cells were centrifuged and the pellet was resuspended and distributed over 4x100mm gelatin-coated plates. 24 hours later, Hypoxanthine Aminopterin Thymidine (HAT, Sigma Aldrich-Aldrich) was added to the culture medium to select cells with successful integration of the targeting vector. After 7-10 days, over which the selection media was changed every 48 hours, HAT resistant colonies were picked into 96-well plates, and maintained in ES medium supplemented with Hypoxanthine-Thymidine (1xHT, Sigma Aldrich). Genomic DNA was extracted and analysed for successful recombination by PCR and southern blots.

Isolation of Genomic DNA

Cell pellets were lysed in 500 μ l of ES cell lysis buffer (10mM Tris pH7.4, 10mM EDTA, 10mM NaCl, and 0.5% Sarkosyl at 30% stock concentration) with freshly added 1mg/ml Proteinase K (20mg/ml stock) and incubated at 55°C O/N. Two phenol chloroform (Sigma Aldrich) extractions were performed using the Maxtract low density tubes (Qiagen) according to manufacturer's instructions. Cleanup of the genomic DNA was done by ethanol precipitation. The DNA pellet was washed with 70% ethanol, re-suspended in milliQ water and stored at -20. Genomic DNA isolated from recombinant ES cell clones was analysed for successful integration of the DNA fragments by qPCR using the following primers: LacZ_F, 5'-GCCATTGTCAGACATGTATAC-3'; LacZ_R, 5'-CTCAACCCTATAGCTCCAG-3'; *Hprt* exon2_F, 5'-GAACCAGGTTATGACCTAGA-3'; *Hprt* exon2_R, 5'-ATTGTGGCCCTCTGTGTGCT-3' (Fig. S2B-D).

Southern blotting

10µg of genomic DNA was digested with HpaI and SacI (NEB) at 37°C O/N. The DNA was separated on a 0.8% agarose gel (1xSYBR safe DNA stain, Invitrogen), run at 25V for 12-14 hours. The gel was subsequently washed with 0.25M HCl solution for 20min, and 0.4M NaOH for 30min. The DNA was then transferred O/N onto a Hybond membrane (Hybond-XL, Amersham) in a 0.4M NaOH solution. The membrane was washed in 0.4M NaOH for 10min, and 2xSSC for 10min. The membrane was then baked in an oven at 85°C for 2 hours to crosslink the DNA followed by incubation in a bottle containing 20ml of hybridisation buffer (0.5M NaP, 7% SDS, 1mM EDTA, and 1% crystalline grade BSA) for 2 hours at 65°C. During this time, the probe was labelled with alpha ³²P-dCTP (PerkinElmer) using a Decaprime II kit (Applied biosystems), as per manufacturers description. The membrane was incubated with the labelled probe at 65°C O/N. Membranes were subsequently washed in 2xSSC at room temperature for 20min, then 0.1xSSC/0.1%SDS preheated to 65°C for 10min. The washed membranes were then developed using a phosphoimager.

ES cell differentiation into EBs

To generate EBs, ES cells were collected and cultured in media consisting of IMDM supplemented with 15% FBS (PAA laboratories) pretested for efficient embryonic stem cell differentiation, 1% L-Glutamine (Invitrogen), 1% penicillin-streptomycin, 0.3% of 0.15M MTG, 0.6% of 30mg/ml transferrin (Life Technologies) and 1% of 5mg/ml ascorbic acid (Sigma Aldrich). Cells were then seeded on ultra-low attachment 60mm plates (Sterilin) and placed in an incubator at 37°C and 5%CO₂. These culture conditions were optimal for growth of day 1-4 EBs ⁵.

LacZ staining

Fluorescein di- β -D-galactopyranoside (FDG, Sigma Aldrich) is a substrate for β -D-galactosidase enzyme that is encoded by the LacZ reporter gene. FDG powder was reconstituted to a 2mM stock solution according to manufacturer's instructions. Cells collected from day 3EB and D2 liquid blast cultures were dissociated and diluted in PBS/2% FBS at a concentration of 3×10^6 cells/0.1ml. On a 37C heat block, FDG was added to the cells at a 1:1 ratio and incubated at 37C for 1 min. The cell suspension was then added to IMDM/20%FBS and placed on ice for 1 hour. Further staining with primary and secondary antibodies was performed after the LacZ staining procedure.

Flow cytometry and cell sorting

Cells were collected from EBs and liquid blast cultures and dissociated into a single cell suspension. Cells were incubated on ice for 30 min either with a biotinylated or fluoro-chrome-conjugated primary antibody. For cells stained with a primary biotinylated antibody this was followed with 30min incubation with SA-pecy7 secondary antibody. Dead cells were excluded from the analysis on the basis of Hoechst 33258 uptake ($1 \mu\text{g/ml}$ final concentration; Invitrogen). The following primary and secondary antibodies were used for staining; TIE2-biotin, TIE2-PE, Endoglin-Biotin, Endoglin-PE, CD117-eFluor780, FLK1-APC, FLK1-Biotin, FLK1-PE, CD41-Biotin, CD41-pecy7, CD41-APC, CD45-percp_cy5.5, CD144-AlexaFluor647, rabbit anti-mouse Lrp2-pecy7 (Bioss antibodies), streptavidin-pecy7 (eBiosciences), and anti-rabbit Alexa Fluor-647 (Invitrogen). Flow cytometry analysis was performed using standard flow cytometers; LSR or BD Canto II. FACS was performed on an Aria II, Influx or Jazz BD.

Methylcellulose blast colony forming (BL-CFC) assay

2×10^4 FLK1+/LacZ⁻ and FLK1+/LacZ⁺ cells sorted from day 3 EBs were seeded in methylcellulose mix which consisted of IMDM supplemented with 55% methylcellulose (BioScientific), 10% FBS pretested for efficient EB differentiation, 1% L-glutamine, 1% L-glutamine, 0.6% of 30mg/ml transferrin, 0.3% of 0.15M MTG, 0.5% of 5mg/ml ascorbic acid, 15% D4T conditioned media, 0.1% of 5 μ g/ml VEGF, and 0.1% of 10 μ g/ml IL-6. A 1ml syringe and 18 gauge blunt-end needle were used to aspirate the methylcellulose mix onto 35mm petri dishes. The blast potential in Flk1+ cells was assayed in triplicate dishes of 1ml. The plates were then placed in a humidified incubator 37°C and 5% CO₂. Colonies were scored following four days of culture ⁵.

Liquid BL-CFC assay

Day 3 EBs were dissociated into single cells using trypsin (Gibco), and stained with a Flk1-PE or Flk1-bio antibody (eBiosciences). Sorted FLK1+ cells were seeded on gelatin coated plates at a density of $7.5-8.5 \times 10^4$ cells/9.6cm² (in a humidified incubator 37°C, 5% CO₂). The culture medium consisted of IMDM supplemented with 10% FBS pre-tested for differentiation, 1% L-Glutamine, 0.6% of 30mg/ml transferrin, 0.3% of 0.15M MTG, 0.5% of 5mg/ml ascorbic acid, 15% D4T conditioned medium, 0.1% of 5 μ g/ml VEGF, and 0.1% of 10 μ g/ml IL-6 (R&D systems).

Hematopoietic methylcellulose colony-forming assay

Cells isolated from day 2 or 4 of liquid blast cultures were seeded in media containing IMDM supplemented with 55% methylcellulose, 15% plasma derived serum (PDS, Animal Technologies), 10% protein-free hybridoma medium (PFHMII, Invitrogen), 1% L-Glutamine, 0.6% of 30mg/ml transferrin, 0.3% of 0.15M MTG, 0.5% of 5mg/ml ascorbic acid, 1% of 10 μ g/ml kit ligand (KL), 0.1% of 1 μ g/ml IL-3, 0.3% of 10 μ g/ml G-CSF, 0.1% of 5 μ g/ml IL-

11, 0.2% erythropoietin at 2000 units/ml, 0.2% of 5µg/ml IL-6, 0.1% of 5µg/ml TPO, and 0.1% of 10µg/ml GM-CSF. Cells were seeded at a concentration of 3x10⁴ cells/ml, and the assay was set up in triplicate dishes of 1ml. Primitive colonies were scored after 4-5 days of culture, whereas most definitive colonies were scored after 7-10 days. All cytokines used in this assay were purchased from R&D systems⁵.

RT-PCR

Prior to RNA isolation from samples, all equipment was treated with RNaseZap (Ambion) to remove any contaminating RNases. RNA was extracted using an RNeasy extraction kit (Qiagen) following manufacturer's instructions. RNA was eluted with 30µl RNase-free water. cDNA was then synthesized using the GoScript Reverse Transcriptase (Promega) following manufacturer's guidelines. The cDNA was analysed for expression using the Express SYBR Green QPCR Supermix universal (Life Technologies). List of primers used for RT-PCR: *Eng*_F, 5'-AAATCCCGTTGCACTTGG-3'; *Eng*_R, 5'-ACTCTTGGCTGTCCTTGGAA-3'; *B-actin*_F, 5'-TGACAGGATGCAGAAGAAGA-3'; *B-actin*_R, 5'-CGCTCAGGAGGAGCAATG-3'; *Lrp2*_F; *Lrp2*_R.

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed as detailed previously³. Briefly, FACS sorted *Bry*-GFP ES cells (+/+ and +/- cell fractions) were treated with 0.4% formaldehyde (Sigma Aldrich) and the cross-linked chromatin was retrieved by nuclei isolation and lysis. The chromatin was sonicated to yield an average fragment size of approximately 500 bp, pre-cleared with rabbit serum (anti-IgG, Millipore), and immunoprecipitated with an anti-acetyl H3K9 antibody (AcH3K9, Millipore) to recover acetylated histones and bound DNA. The DNA was then purified, and enrichment at the *Eng* locus was measured by real-time PCR. Expression levels

were normalized to *Eng* -4 (Negative control region). List of primers used to quantify enrichment; Eng-8_F, 5'-TGTCATTGTCTTCTGGTCTC-3'; Eng-8_R, 5'-ACACTCTCTGGGCATAGC-3'; Eng-4_F, 5'-AAGCGGCATTGGATATTG-3'; Eng-4_R, 5'-AAGGTTAGGTTTCGTTTGG-3'; EngP_F, 5'-ACTTCTCCTGACTTCTCC-3'; EngP_R, 5'-CGGTATCCAGAGGTAAGG-3'; Eng+7_F, 5'-CCAGAGCAACTTGATGAC-3'; Eng+7_R, 5'-TACTCCTCCTCCTCCTTC-3'; Eng+9_F, 5'-TGGGTCAGGGTGAAATTCC-3'; Eng+9_R, 5'-AAGGCCGGTGATGTAGAGC-3'.

Mouse embryo immunostaining and imaging

10um thick cryosections of E10.5 fixed mouse embryos embedded in a gelation/sucrose solution were thawed and rehydrated in PBS prior to immunostaining. The sections were streptavidin/biotin blocked followed by serum blocking (PBS with 10% FCS, 0.05% Tween20 and of 10% goat serum (DAKO) for 1 hour before the sections were incubated with primary antibodies at 4°C overnight in blocking buffer. Immunostaining of E10.5 embryo sections was performed using purified rat anti mouse CD31 (553370, MEC13.3, BD Biosciences) (1/100) and guinea pig anti-mouse Lrp2 primary antibody (Dr Annette Hammes, Max-Delbrueck-Center for Molecular Medicine, Berlin). Sections were washed three times in PBST for 15 minutes each and then incubated with fluorochrome-conjugated secondary antibodies Alexa Fluor(r) 488 Goat Anti-Rat IgG (A11006, Life Technologies) and Alexa Fluor(r) 647 goat anti-guinea pig IgG (A-21450, Life Technologies) at a 1/400 dilution. Sections were further washed three times in PBS and mounted using Prolong Gold antifade reagent with DAPI (Life Technologies). Images were taken using a low-light time lapse microscope (Leica) using the Metamorph imaging software and processed using ImageJ.

Generating zebrafish morpholinos and analysis

Morpholinos against *lrp2a* (5'-AATCAGTGCTTGTGGTTTACCTGGG-3',⁶), *lrp2b* (5'-CACCCTCATGCACTGACCTGACCTGCACA-3',⁷) and *p53* (5'-GCGCCATTGCTTTGCAAGAATTG-3',⁸) were purchased from Gene Tools. *Lrp2a/b* morpholinos were injected at increasing doses of 1ng, 2ng and 4ng each. The *p53* morpholino was injected at a previously determined optimal dose of 4ng (Zon lab). The *Lrp2* rescue was performed using a Human *lrp2* vector obtained from Origene. Plasmid was linearized with *Sfo1* and *in-vitro* transcribed using mMessage mMACHINE T7 kit (Thermo Fisher). The mRNA was injected at two different doses "low" (20pg/embryo) and "high" (100pg/embryo). Embryos were fixed at 36hpf followed by analysis of *runx1* and *cmyb* expression in the AGM which was analyzed by in situ hybridization (as previously described⁹). Zebrafish embryo images were taken with a Leica stereoscope. For the analysis of vascular integrity *flk:zsgreen* embryos were injected with the lowest effective MO dose of 2ng of each *lrp2* morpholino (*lrp2a* and *lrp2b*) as determined by in situ hybridization. Embryos were aged to 32h. Ten embryos for each condition were anesthetized in 0.04 mg/ml Tricaine, mounted in 1% low-melting agarose and imaged with a Leica stereoscope. A high-resolution image for a representative embryo of each group was acquired on a Zeiss confocal microscope.

Analysis of adult peripheral blood and bone marrow

Blood samples were diluted in PBS and analyzed using an Ac · T Diff analyzer (Beckman Coulter) using equine setting. Suspensions of BM cells from the femurs were isolated and depleted of red blood cells by an ammonium chloride lysis step (STEMCELL Technologies, STEMCELL)). HSC and progenitor fractions were identified using the following antibodies: CD45-FITC or APC-Cy7 (Clone 30-F11 Biolegend), EPCR-PE (Clone RMEPCR1560, STEMCELL), CD150-Pacific Blue or PE-Cy7 (Clone TC15-12F12.2, Biolegend), CD48-APC (Clone HM48-1, Biolegend), Sca-1-Pacific Blue or PE (Clone E13-161.7, Biolegend),

FLT3-PE or PE-Cy5 (Clone A2F10, eBioscience), CD34-FITC (Clone RAM34, BD Biosciences), c-kit APC-Cy7 (Clone 2B8, Biolegend), and a panel of lineage markers (Hematopoietic Progenitor Enrichment Cocktail, STEMCELL) plus streptavidinV500 (BD Biosciences). See ¹⁰ for details.

Statistical analysis

RT-PCR data, BL-CFCs and hematopoietic colony counts were statistically analysed using Student's T-test or Paired Student's T-test. Significant differences are marked * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$ and not significant differences are marked NS.

RNA sequencing and analysis

Cells were sorted into IMDM/20%FCS and centrifuged. The cells were lysed in RNA lysis buffer/1% β -mercaptoethanol and snap frozen in liquid nitrogen. RNA was isolated from cells as per standard method (For details about samples refer to supplementary table S3), and amplified using the Ovation RNA amplification system V2 (Nugen). Single-stranded TruSeq cDNA libraries were generated and sequenced using the Illumina HiSeq2000 analyzer (BGI, Hong Kong). The sequencing reads were aligned to the mouse genome (GRCm38/mm10) using the software package TopHat. Transcript expression for FLK1+/LacZ- and FLK1+/LacZ+ samples sorted from day 3 EBs was quantified with the software suite HOMER using standard parameters and two characteristically different bioinformatics tools, GFOLD and DeSeq, were used to identify differentially expressed genes. GFOLD was used to identify genes with large fold changes (e.g. within the 1% and 99% percentiles of GFOLD estimates), and DeSeq was used to identify genes that significantly changed across sample populations (≤ 0.05). Samples were collected in triplicates, and each experiment was analysed independently to generate a list of DEGs. Heat maps were generated using the R package

pheatmap [pheatmap: Pretty Heatmaps. R package version 061]. Patterns were generated based on normalized FPKM, with expression levels lower than 1/3 assigned as low (L), between 1/3 and 2/3 as intermediate (I), and more than 2/3 as high (H). Gene lists from each resulting category were analysed using Panther gene ontology program. The data has been deposited in GEO under the accession number GSE77390.

Pathway and Gene-set enrichment analysis

The Ingenuity IPA Core Analysis Tool (version 17199142) was used to establish 'Molecular and Cellular Functions' that correlate with the lists of differentially expressed genes. Gene-set enrichment analysis was performed using the GSEA Java Desktop tool (v 2.0.13). Gene-expression levels were obtained from RNA-seq, and ranked using the GFOLD algorithm. The GSEA pre-ranked tool was used to interrogate the enrichment of various biological pathways provided by publically available databases such as Kegg and Biocarta. Other datasets were obtained from the GEO NCBI website; Ldb1 WT vs. Ldb1^{-/-} (Accession number: GSE43044), Etv2^{+/-} vs. Etv2^{-/-} (Accession number: GSE31743), EC vs. HEC, and HEC vs. HSC (Data from ¹¹ provided by Professor Elaine Dzierzak).

SUPPLEMENTAL REFERENCES

1. Fehling HJ, Lacaud G, Kubo A, et al. Tracking mesoderm induction and its specification to the hemangioblast during embryonic stem cell differentiation. *Development*. 2003;130(17):4217-4227.
2. Magin TM, McWhir J, Melton DW. A new mouse embryonic stem cell line with good germ line contribution and gene targeting frequency. *Nucleic Acids Res*. 1992;20(14):3795-3796.
3. Pimanda JE, Chan WY, Donaldson IJ, Bowen M, Green AR, Gottgens B. Endoglin expression in the endothelium is regulated by Fli-1, Erg, and Elf-1 acting on the promoter and a -8-kb enhancer. *Blood*. 2006;107(12):4737-4745.
4. Pimanda JE, Chan WY, Wilson NK, et al. Endoglin expression in blood and endothelium is differentially regulated by modular assembly of the Ets/Gata hemangioblast code. *Blood*. 2008;112(12):4512-4522.
5. Sroczyńska P, Lancrin C, Kouskoff V, Lacaud G. The differential activities of Runx1 promoters define milestones during embryonic hematopoiesis. *Blood*. 2009;114(26):5279-5289.
6. Anzenberger U, Bit-Avragim N, Rohr S, et al. Elucidation of megalin/LRP2-dependent endocytic transport processes in the larval zebrafish pronephros. *J Cell Sci*. 2006;119(Pt 10):2127-2137.
7. Kur E, Christa A, Veth KN, et al. Loss of Lrp2 in zebrafish disrupts pronephric tubular clearance but not forebrain development. *Dev Dyn*. 2011;240(6):1567-1577.
8. Langheinrich U, Hennen E, Stott G, Vacun G. Zebrafish as a model organism for the identification and characterization of drugs and genes affecting p53 signaling. *Curr Biol*. 2002;12(23):2023-2028.
9. Thisse C, Thisse B. High-resolution in situ hybridization to whole-mount zebrafish embryos. *Nat Protoc*. 2008;3(1):59-69.
10. Wilson NK, Kent DG, Buettner F, et al. Combined Single-Cell Functional and Gene Expression Analysis Resolves Heterogeneity within Stem Cell Populations. *Cell Stem Cell*. 2015;16(6):712-724.
11. Solaimani Kartalaei P, Yamada-Inagawa T, Vink CS, et al. Whole-transcriptome analysis of endothelial to hematopoietic stem cell transition reveals a requirement for Gpr56 in HSC generation. *J Exp Med*. 2015;212(1):93-106.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1 Characteristics and functional behaviour of Endoglin regulatory elements.

(A) Table listing combinations of *Eng* regulatory elements tested in mouse transgenic reporter assays {Pimanda, 2008 #621}. Tissues with hematopoietic and endothelial activity; placenta, yolk sac (YS), dorsal aorta (DA), fetal liver (FL) and heart tissue were investigated for *lacZ* reporter gene expression in endothelial (E) and hematopoietic (H) cells. (B) Table listing the mouse/human coordinates and size of the *Eng* -8, P, +7, +9, +7Δ, +9Δ regulatory elements {Pimanda, 2008 #621}. (C) Table listing the combinations of the *Eng* enhancer-driven LacZ reporter gene constructs which were used in this study.

Figure S2. Generating recombinant mouse ES cell lines using combinations of the LacZ/Eng regulatory elements.

(A) Schematic representation of the *Hprt* targeting strategy. Reporter constructs were cloned into the pMP8 plasmid between the 5'-3' *Hprt* homology arms. After electroporation into HM1 ES cells, the respective 5'-3' sites recombined and integrated the construct into the genome. The *Eng* -8/P/*LacZ* construct was used as an example in this figure. (B) Schematic of the recombinant *Hprt* locus. Successful recombination was verified by PCR and southern blots. (C) PCR#1 verified the integration of the targeting construct into the genome. Size of the PCR product varied from 1-2Kb depending on the type of insert. (i) Size of the PCR product for *Eng*-8/P ES cells was 1Kb. (ii) Size of PCR product for *Eng*-8/P/*LacZ*/+7 or +9 was 1.5Kb. (iii) Size of PCR product for *Eng*-8/P/*LacZ*/+7/+9 was 2Kb. (iv) Size of PCR product for *Eng*-8/P/*LacZ*/+7Δ/+9 and *Eng*-8/P/*LacZ*/+7Δ/+9Δ was 2Kb. The positive control for each PCR was a pMP8 plasmid containing the corresponding construct. (D) PCR#2 verified the integration of the construct specifically into the *Hprt* locus. The PCR spanned from exon 2 in the targeting construct, to exon 3 in the endogenous *Hprt* locus. A 3.2Kb PCR product was detected for all successfully

recombinant clones. No PCR product was detected for HM1 ES cells which lack exons 1 and 2. **(E)** The southern blot analysis quantified copy number integration. The probe was a 250bp fragment that binds to a region between exons 3 and 4 of the *Hprt* locus. We detected a 6.5Kb band for the successfully recombinant clones, and a 9Kb band for HM1 ES cells. The white boxes highlight the southern blot band for each recombinant ES cell line tested.

Figure S3. Bar charts showing BL-CFC numbers/3000 seeded cells sorted from day 3 EBs generated from recombinant ES cells. Experiments were performed in triplicate and repeated two times. Statistical analysis using students t-test, *P<0.05; **P<0.01; ***P<0.001; NS, not significant.

Figure S4. RNA-sequencing comparative analysis of FLK1+/LacZ- and FLK1+/LacZ+ sorted fractions. **(A)** List of up- and down-regulated receptors and transcription factors in the FLK1+/LacZ+ cell population. **(B)** Gene network diagram showing genes upregulated in the FLK1+/LacZ+ fraction that are associated with the angiogenic and vasculogenic development network. **(C)** Bar chart showing a list canonical pathways with highest p-values associated with genes up-regulated in the FLK1+/LacZ+ fraction. **(D)** Bar chart showing diseases and biological functions with highest p-value associated with genes up-regulated in the FLK1+/LacZ+ fraction. **(E)** Bar chart showing a list canonical pathways with highest p-values associated with genes down-regulated in the FLK1+/LacZ+ fraction. **(F)** Bar chart showing diseases and biological functions with highest p-value associated with genes down-regulated in the FLK1+/LacZ+ fraction.

Figure S5. -8/P/LacZ/+7Δ/+9, and -8/P/LacZ/+7Δ/+9Δ mutant constructs do not enrich cells with hemogenic potential in HEI. At day 2 of liquid blast cultures, HEI LacZ⁺/LacZ⁻ fractions were sorted and re-seeded in liquid blast media for 2 days and then seeded into CFU-C assays. **(A)** Bar chart showing the number of hematopoietic colonies generated from Eng -8/P/LacZ/+7Δ/+9 ES cells. **(B)** Bar chart showing the number of hematopoietic colonies generated from Eng -8/P/LacZ/+7Δ/+9Δ ES cells. 3×10^4 cells were seeded in methylcellulose mix. Primitive and definitive colonies were counted after four and nine days respectively. Experiments were performed in triplicate and repeated two times. Statistical analysis using students t-test, *P<0.05; **P<0.01; ***P<0.001; NS, not significant.

Figure S6. RNA-sequencing analysis of ENG⁺/LacZ⁻, ENG⁺/LacZ⁺ and ENG⁻/LacZ⁺ HEI fractions sorted from day 2 liquid blast cultures. **(A)** GSEA plots showing the overlap of genes that are differentially expressed between ENG⁺/LacZ⁻ and ENG⁻/LacZ⁺ HEI and genes in the hypoxia via HIF1a pathway (upper panel) and cell cycle regulators (lower panel). **(B)** Network diagram of genes that are associated with hematopoietic development and function and are upregulated in ENG⁻/LacZ⁺ HEI cells. **(C-E)** Differentially expressed genes within each cell fraction were further analysed based on their relative expression pattern across the three HEI populations. The FPKM values for each gene were normalized across the three populations, which resulted in a specific expression pattern. H=High, I=Intermediate, L=Low. The genes within each fraction were further analysed in PANTHER for their association with GO biological functions. **(C)** Analysis of genes differentially expressed in the ENG⁺/LacZ⁻ HEI fraction. (i) Histograms displaying the expression pattern of genes in the HLL, HIL, HHL, and HHI. (ii) Table listing the biological functions associated with genes in each group. **(D)** Analysis of genes differentially expressed in the ENG⁺/LacZ⁺ fraction. (i) Histograms displaying the expression pattern of genes in the

HHL, HHI, and IHL. (ii) Table listing the biological functions associated with genes in each group. (E) Analysis of genes differentially expressed in the ENG-/LacZ⁺ fraction. (i) Histograms displaying the expression pattern of genes in the LLH, IHH, LIH, and ILH. (ii) Table listing the biological functions associated with genes in each group.

Figure S7. Functional and regulatory aspects of genes shared between FLK1⁺/LacZ⁺ cells from day 3 EBs and ENG-/LacZ⁺ HEI cells. (A) (i) List of common genes categorized into genes upregulated in both cell fractions (green), genes downregulated in the FLK1⁺/LacZ⁺ cells and upregulated in the ENG-/LacZ⁺ HEI (red), genes upregulated in the FLK1⁺/LacZ⁺ cells and downregulated in the ENG-/LacZ⁺ HEI (yellow). (ii) Gene regulatory network association between all three gene sets. (iii) The genes listed in table S6A were further analysed based on their relative expression pattern across the FLK1⁺/LacZ⁻, FLK1⁺/LacZ⁺, ENG⁺/LacZ⁻ HEI, ENG⁺/LacZ⁺ HEI, and ENG-/LacZ⁺ HEI populations. (i) The FPKM values for each gene were normalised across all populations, which resulted in a specific expression pattern. H=High, I=Intermediate, L=Low (ii) Each gene set was analysed using genes2Canvas for their association with specific cell types. The canvas associated with each gene set was overlaid. (B) Bar chart showing the molecular and cellular functions associated with each gene set. (C) Table listing genes that were up-regulated in FLK1 mesodermal (FLK1⁺/lacZ) and HE1 cells (ENG-/lacZ) that were targeted by *Eng* regulatory elements with a detailed description of their known functions and relation to hematopoietic development if known.

Figure S8. Genes down-regulated in both FLK1⁺/LacZ⁺ cells from day 3 EBs and ENG- /LacZ⁺ HEI cells. (A) Venn diagram showing the overlap and identity of genes that were down-regulated in FLK1 mesodermal (FLK1⁺/lacZ) and HE1 cells (ENG-/lacZ) cells. (B)

Table listing genes from (A) with a detailed description of their known functions and relation to hematopoietic development if known.

Figure S9. Lrp2 a/b and tp53 morpholinos. (A) Analysis of WT and *lrp2a/b* MO zebrafish for the presence of HSCs at 36 hpf following MO injections at 24 hpf. Low (left panel) and high (right panel) magnifications are shown and numbers in the panels indicate the number of embryos with the depicted phenotype. ISH for the HSC marker *runx1*. (B) ISH for *cmyb* following in *lrp2a/b* morphants co-injected with *tp53*MO to exclude non-specific cell death.

Figure S10. LRP2 null FVB/N adult mice (A) Peripheral blood counts (Mean (SD)) from 9 month old LRP2KO mice and littermates. (B) Bone marrow flowcytometry based assessment of MNC fractions. Differences were not significant.

A







Endoglin transgenic construct	Placenta	YS	DA	Liver		Heart	Reference
-8/P/LacZ	No	E	E	E	medium H	E	Pimanda et al Blood 2006 Jun 15;107(12)
-8/P/LacZ/+7	No	E	E	E	High H	E	Pimanda et al Blood. 2008 Dec 1;112(12)
-8/P/LacZ/+9	No	E	E	E	medium H	E	Pimanda et al Blood. 2008 Dec 1;112(12)
-8/P/LacZ/+7/+9	E	E	N/A	E	High H	E	Pimanda et al Blood. 2008 Dec 1;112(12)

E=Endothelial
H=Hematopoietic
No=No staining
N/A=No observed

B

Regulatory elements	Mouse sequence coordinates (mm7)	Size (bp)	Reference	Human sequence coordinates (hg18)
<i>Eng</i> -8	Chr2:32645367-32645659	329	Pimanda et al Blood 2006 Jun 15;107(12)	Chr9:129664306-129664634
<i>Eng</i> P	Chr2:32653786-32654245	484	Pimanda et al Blood 2006 Jun 15;107(12)	Chr9:129656479-129656962
<i>Eng</i> +7	Chr2:32661915-32662416	483	Pimanda et al Blood. 2008 Dec 1;112(12)	Chr9:129648543-129649025
<i>Eng</i> +9	Chr2:32663000-32663504	500	Pimanda et al Blood. 2008 Dec 1;112(12)	Chr9:129646983-129647482
<i>Eng</i> +9Δ	Δ Gata sites	453	Pimanda et al Blood. 2008 Dec 1;112(12)	
<i>Eng</i> +7Δ	Δ Ets sites	374	Pimanda et al Blood. 2008 Dec 1;112(12)	

C

Construct	Schematic
-8/P	
-8/P/+7	
-8/P/+9	
-8/P/+7/+9	
-8/P/+7Δ/+9	
-8/P/+7Δ/+9Δ	

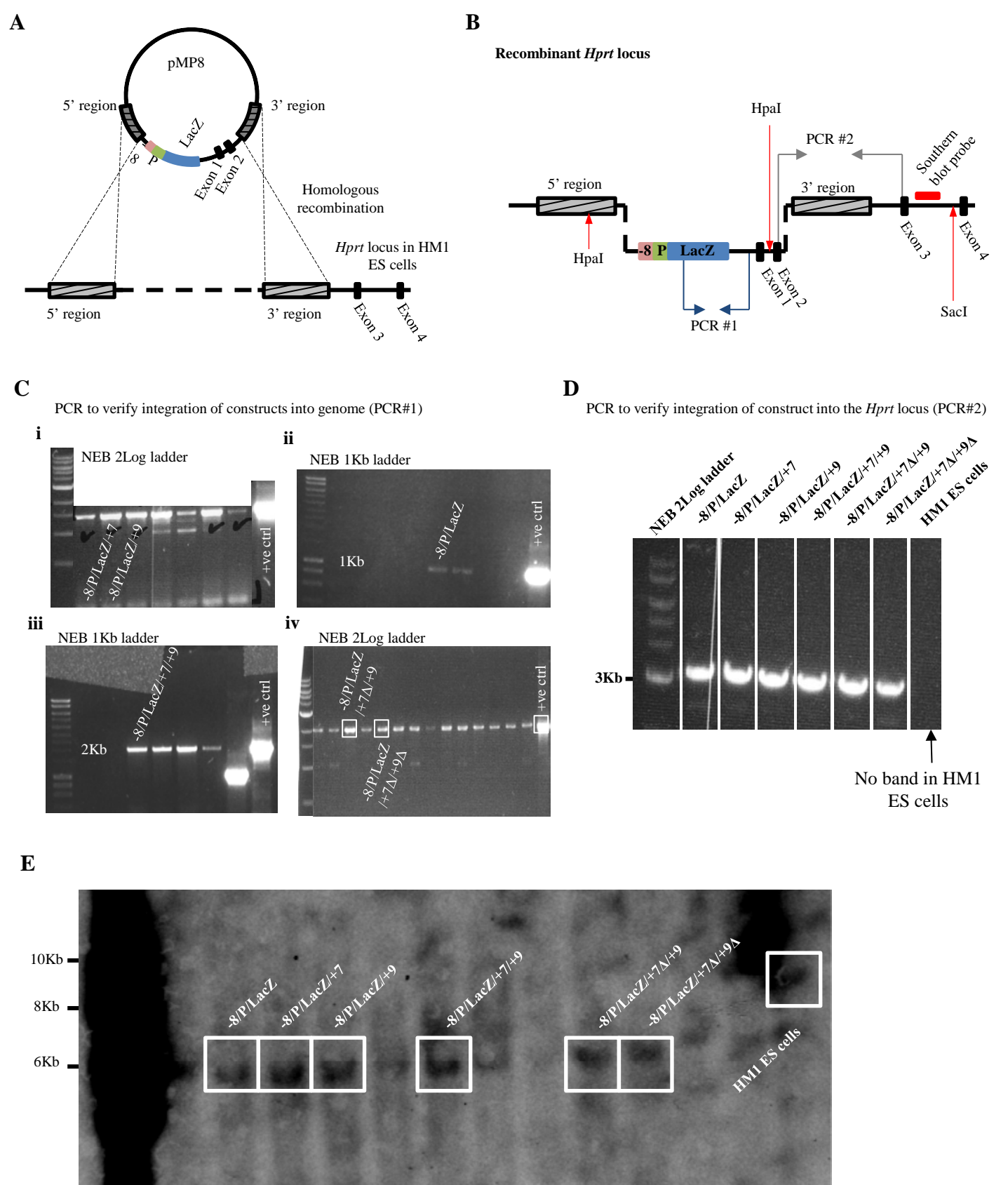
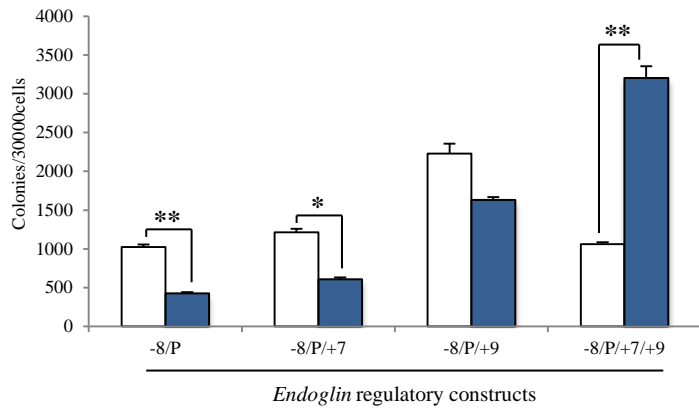


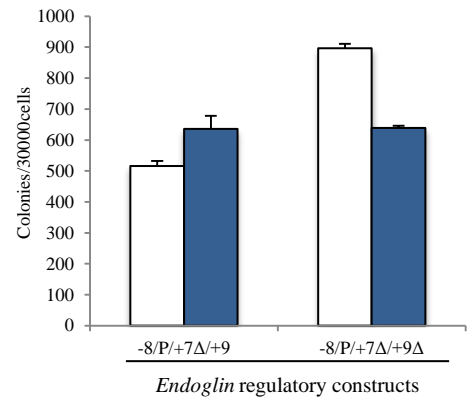
Figure S2

A

Blast colonies

**B**

Blast colonies



A

Up regulated cell surface receptors	LogFC	Upregulated transcription factors	LogFC
Disp2	5.774	Utf1	4.471
Cldn5	5.657	Mecom	3.468
Tlr4	4.681	Ldb2	3.166
Ptchd1	4.602	Gfi1b	2.611
Cdh5	3.583	Hoxb5	2.543
Tie1	3.352	Lyl1	2.382
Ednrb	3.351	Sox7	2.182
Esam	3.165	Erg	2.127
Cd93	2.813	Bcl6b	2.082
Pecam1	2.753	Cbfa2t3	1.968
Cd34	2.725	Fli1	1.891
Icam2	2.471	Sox18	1.837
Tek	2.420	Ptrf	1.690
Flt4	1.852	Klf2	1.543

Down regulated cell surface receptors	LogFC	Down regulated transcription factors	LogFC
Cxcr2	-3.137	Churc1	-3.228
H2-T23	-3.035	Znhit3	-1.523
Lepr	-2.193	Pfdn5	-1.435
Ppp1r16b	-2.167	Lhx1	-0.961

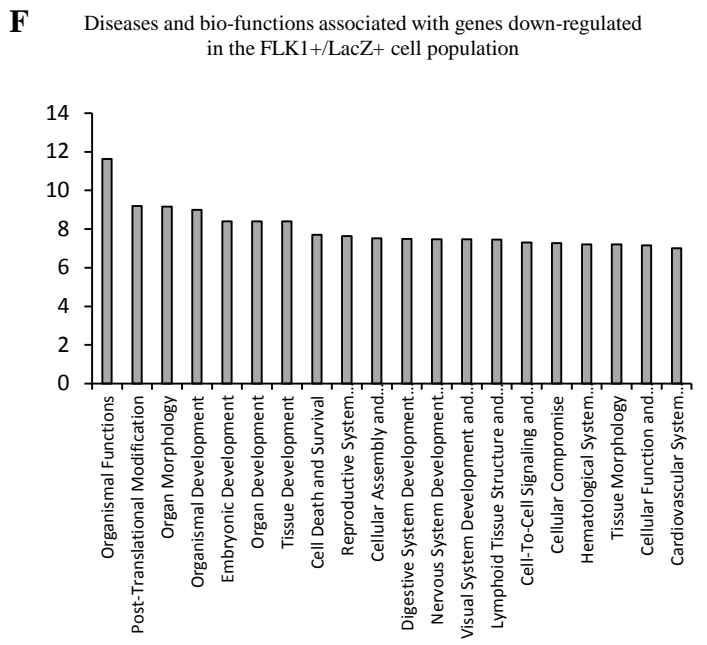
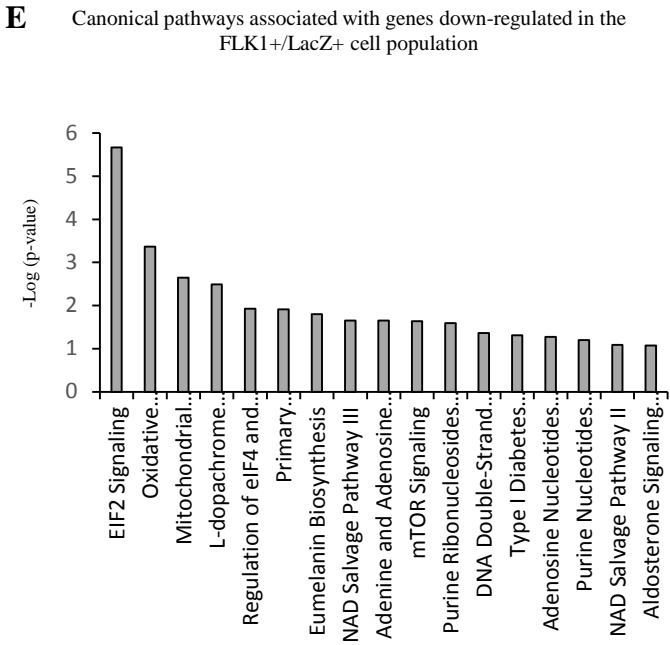
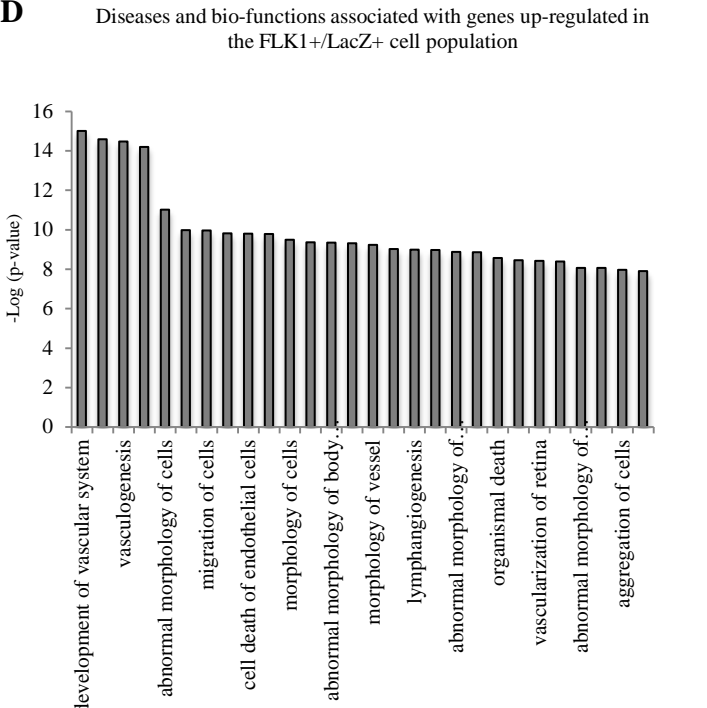
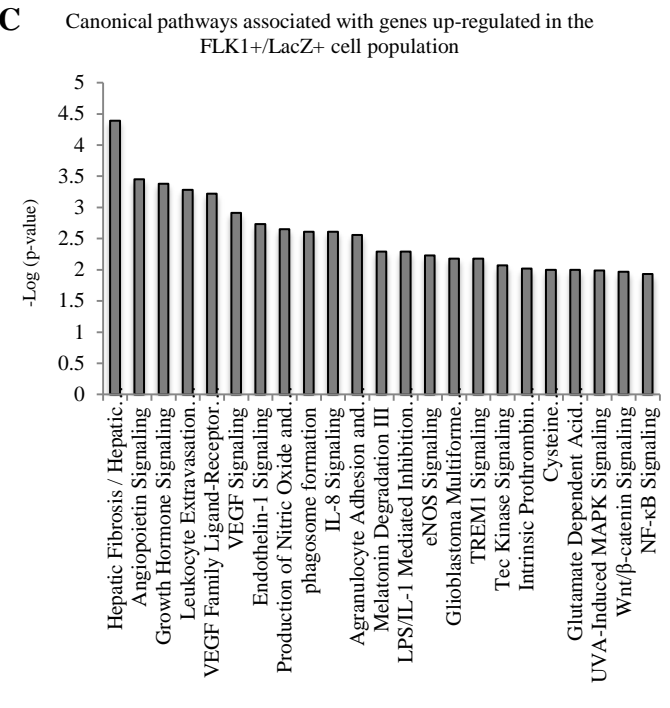
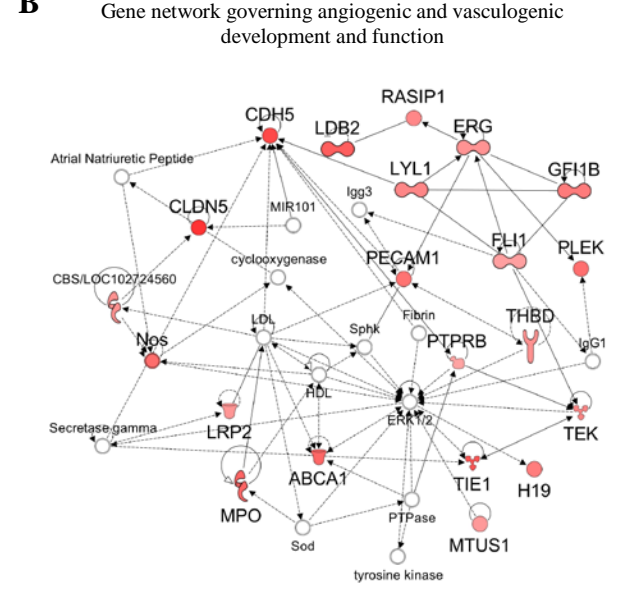
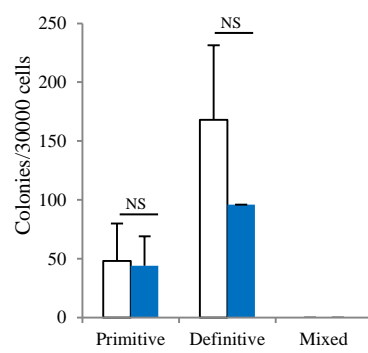
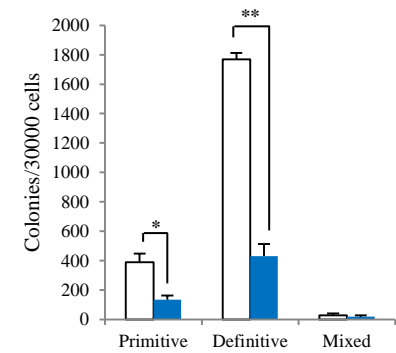
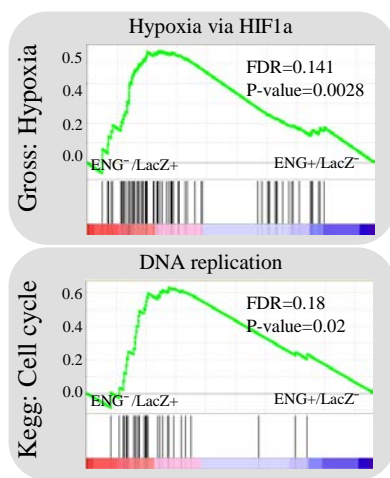
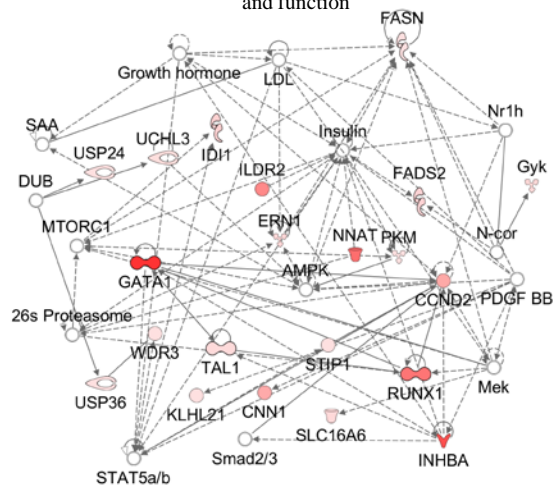
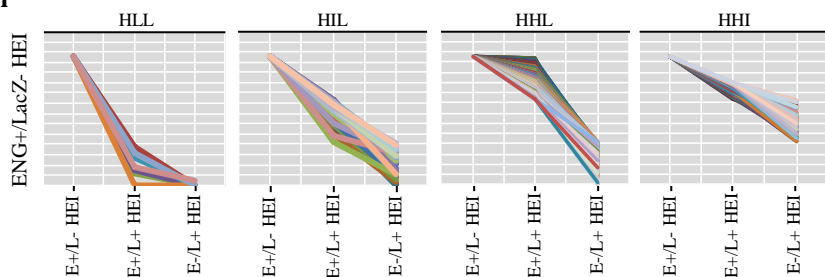


Figure S4

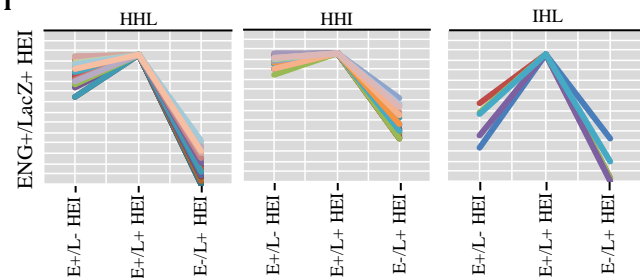


A**B**

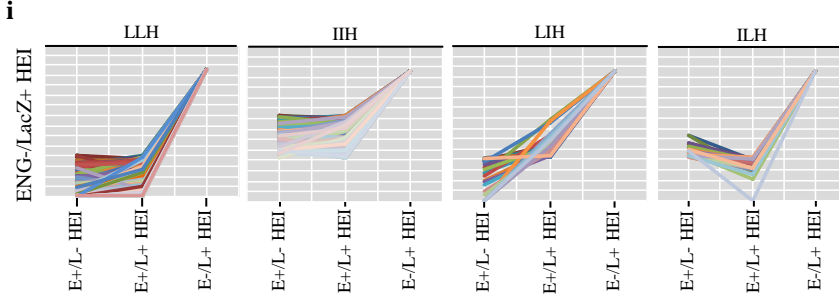
Gene network governing hematopoietic development and function

**C****ii**

Gene Pattern	GO biological process complete	Log (p-value)
HHL	tube morphogenesis	10.7
	epithelial tube morphogenesis	9.9
	regulation of angiogenesis	9.5
	regulation of vasculature development	9.5
HHI	regulation of Ras protein signal transduction	9.1
	negative regulation of catabolic process	8.9
	muscle organ morphogenesis	8.4
	regulation of small GTPase mediated signal transduction	8.3
HIL	macromolecule metabolic process	6.9
	positive regulation of biosynthetic process	6.4
	positive regulation of cellular biosynthetic process	6.4
	regulation of actin filament-based process	5.8

D**ii**

Gene pattern	GO biological process complete	Log (p-value)
HHL	positive regulation of developmental process	5.7
	regulation of developmental process	5.7
	regulation of cell differentiation	4.9
	regulation of localization	4.8
	negative regulation of cell differentiation	4.8
	negative regulation of developmental process	4.8
HHI-IHL	developmental process	5.3
	system development	5.0
	protein metabolic process	4.9
	cellular process	4.8
	regulation of molecular function	4.5
regulation of catalytic activity	4.5	

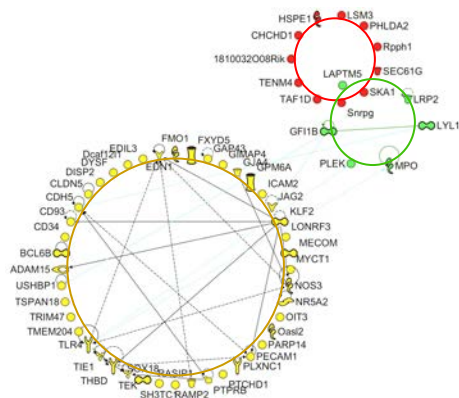
E**ii**

Gene pattern	GO biological processes	Log (p-value)
LLH	positive regulation of immune system process	11.7
	myeloid cell differentiation	9.9
	immune system development	9.8
	hematopoietic or lymphoid organ development	9.8
	leukocyte differentiation	7.4
IHH	organ development	7.03
	cellular macromolecule localization	6.9
	establishment of localization	5.4
LIH	single-organism transport	5.4
	regulation of protein phosphorylation	5.3
ILH	regulation of cell migration	5.3
	regulation of cell motility	4.9

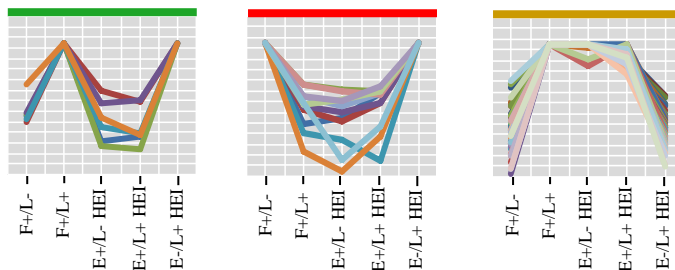


Common genes between D3 EB and D2 HE			
Genes Up in D3 EB and D2 HE	Gfi1b	Adam15	Mecom
	Laptm5	Bcl6b	Myct1
	Lrp2	Cd34	Nos3
	Lyl1	Cd93	Nr5a2
	Mpo	Cdh5	Oas12
	Plek	Cldn5	Oit3
		Dcaf12l1	Patp14
		Disp2	Pecam1
		Dysf	Plxnc1
		Edil3	Ptchd1
Genes Down in D3 EB and up in D2 HE	Edn1	Ptprb	
	Elt1	Ramp2	
	1810032O08Rik	Fmo1	Rasip1
	Chchd1	Fxyd5	Sh3tc1
	Hspe1	Gap43	Sox18
	Lsm3	Gimap4	Tek
	Phlda2	Gja4	Thbd
	Rpph1	Gpm6a	Tie1
	Sec61g	Icam2	Tlr4
	Ska1	Jag2	Tmem204
Snrpg	Klf2	Trim47	
Taf1d	Lonrf3	Tspan18	
Tenm4		Ushbp1	

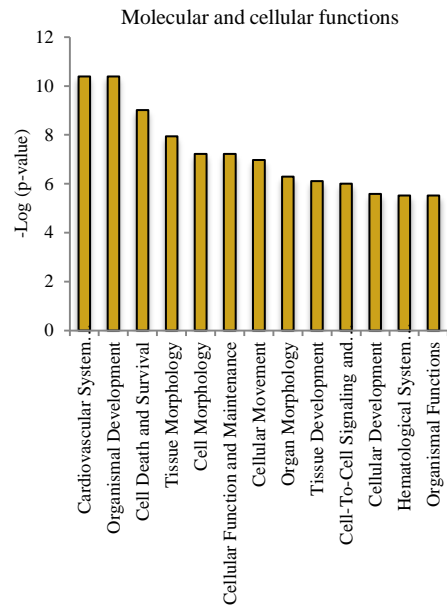
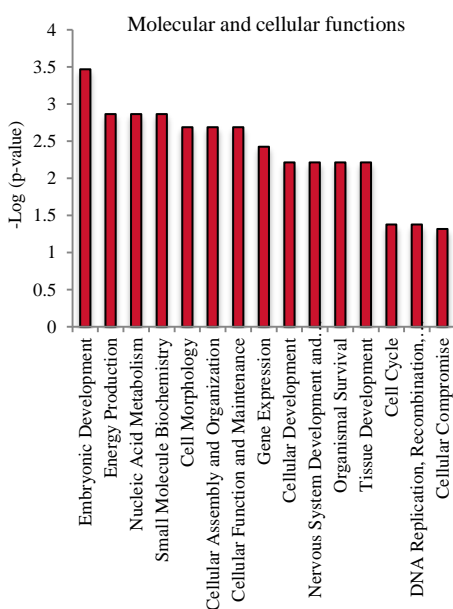
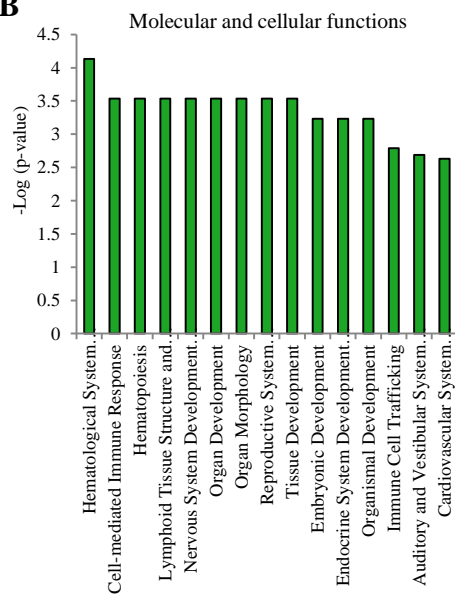
ii



iii



B

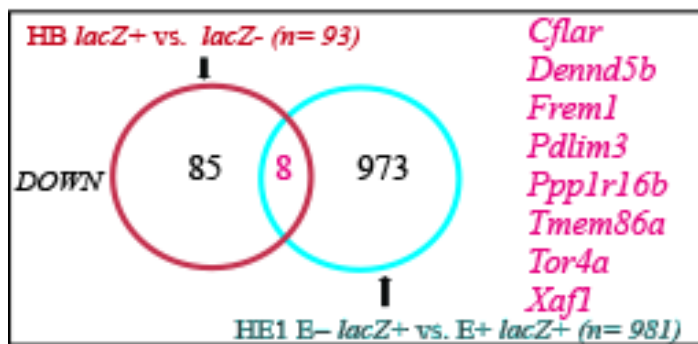


C

UP in D3 EB and UP D2 HE			
Gene	Entrez Gene Name	Disease or function	Associated with hematopoiesis
Gfi1b	growth factor independent 1B transcription repressor	Development of blood, bone marrow, cardiovascular system, connective tissue, embryonic stem cells, hematopoiesis in embryo, hematopoietic progenitor cells, hematopoietic system and tissue, differentiation and maturation of blood and BM.	Yes
Laptm5	lysosomal protein transmembrane 5	Activation and proliferation of blood cells (leukocytes, lymphocytes, T lymphocytes), delayed hypersensitive reaction.	Yes
Lrp2	low density lipoprotein receptor-related protein 2	Abnormal morphology of embryonic tissue, brain, CNS, embryonic body, telencephalic vesicles, loss of neurons, cancer, conventional melanoma	No
Lyl1	lymphoblastic leukemia associated hematopoiesis regulator 1	angiogenesis, apoptosis of blood and hematopoietic progenitor cells, development of blood cells, differentiation and function of hematopoietic progenitor cells, hematopoiesis of bone marrow, T cell development, thrombocytopenia	Yes
Mpo	myeloperoxidase	Abnormal metabolism, function of cardiovascular system, function and movement of blood cells, inflammatory response,	Yes
Plek	pleckstrin	Activation and aggregation of blood cells, coagulation, cytopenia, hemorrhagic disease, hemostasis, thrombocytopenia,	Yes

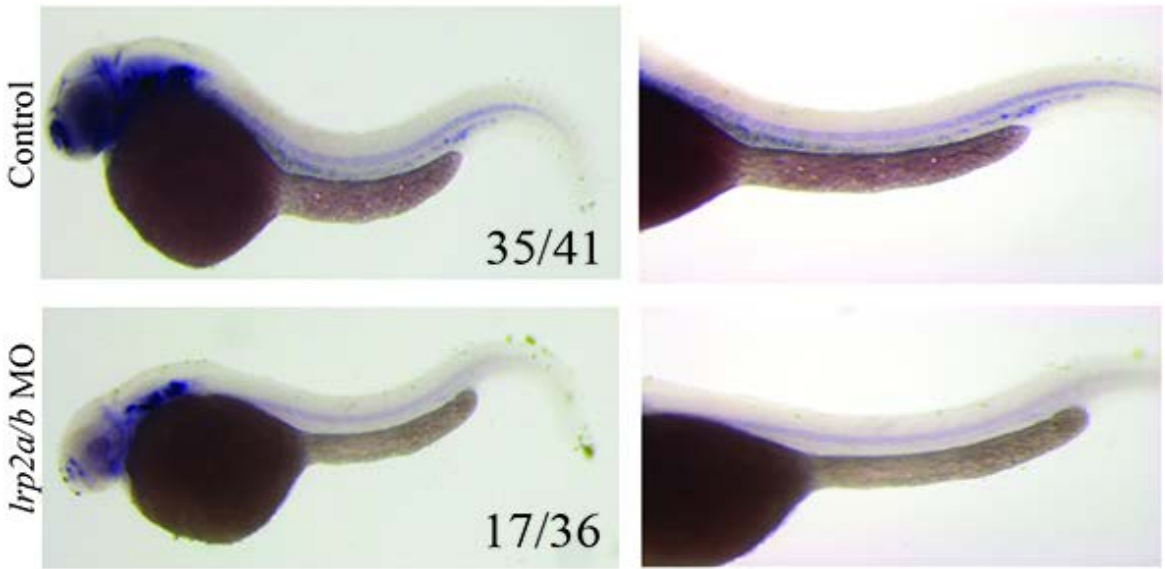
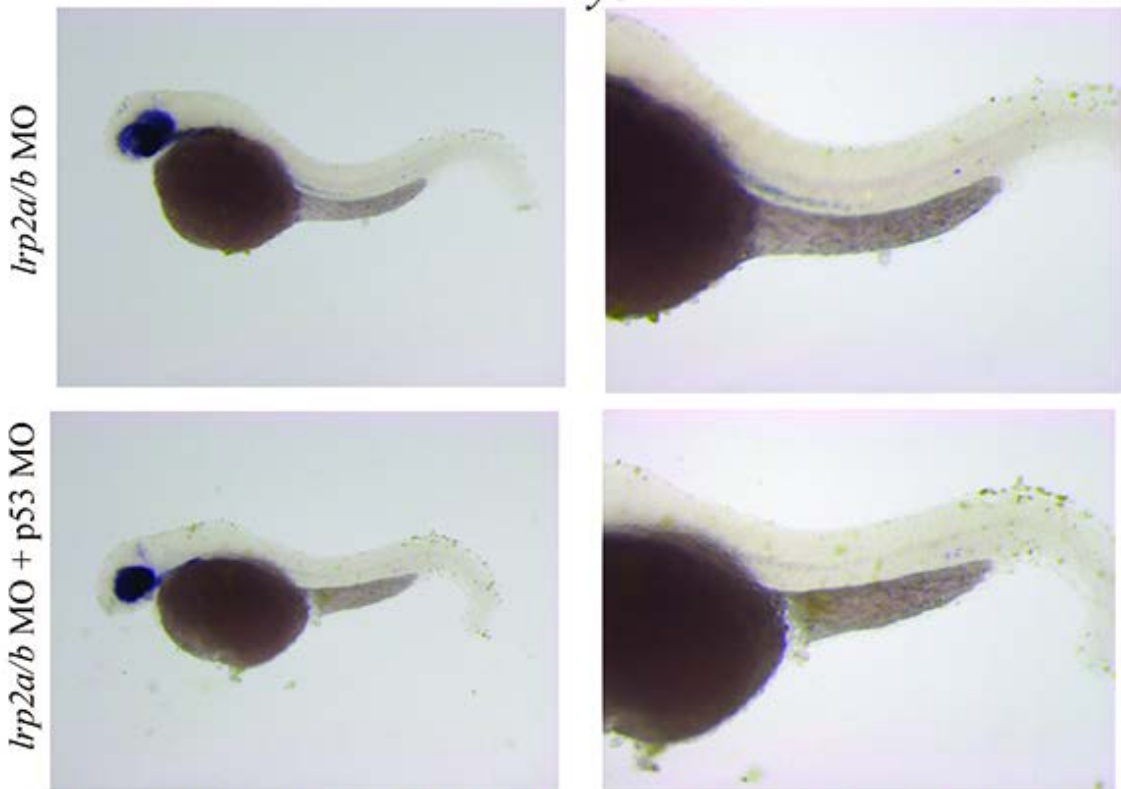
Figure S7

A



B

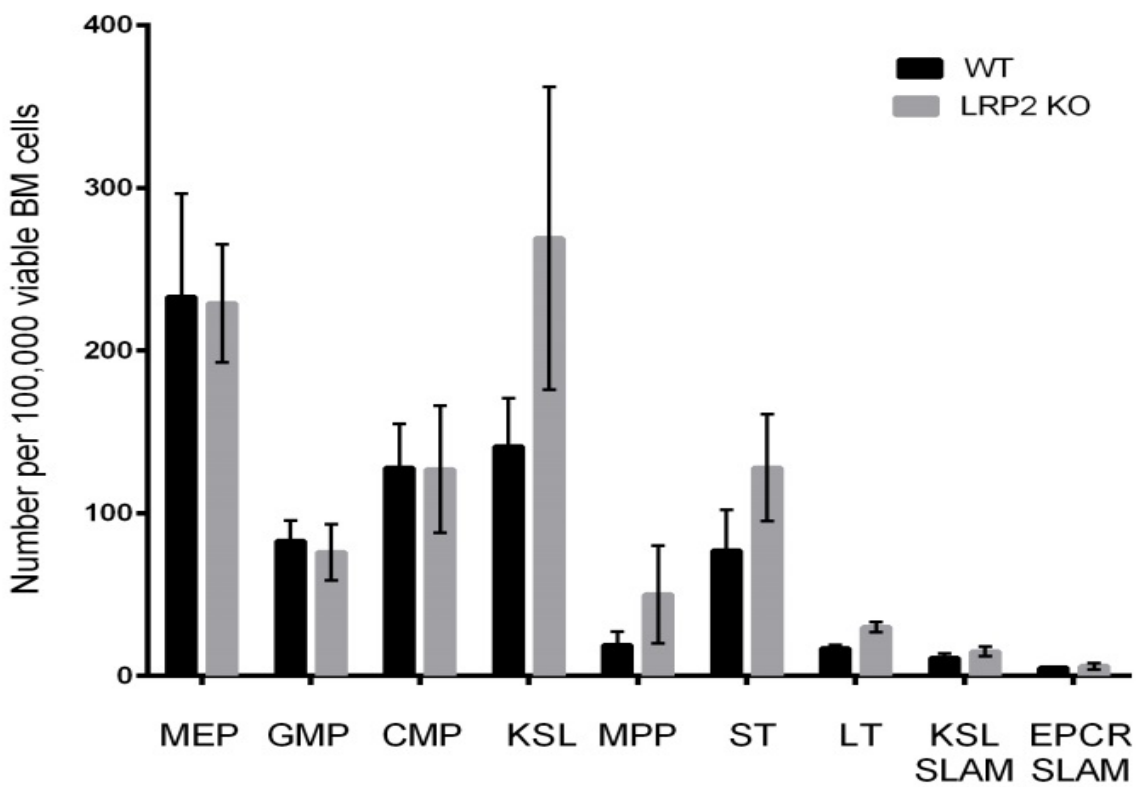
DOWN in D3 EB and DOWN in D2 HE			
Gene	Entrez Gene Name	Disease or function	Association with hematopoiesis
<i>Pdlim3</i>	PDZ and LIM domain protein 3	Transcription factor (non-motor actin binding protein)	No
<i>Tmem86a</i>	Lysoplasmalogenase-like protein TMEM86 A		Expressed in hematopoietic stem cells of the lateral plate mesoderm and cardiovascular system
<i>Cflar</i>	CASP8 and FADD-like apoptosis regulator	Cysteine protease	Widely expressed. Higher expression in skeletal muscle, pancreas, heart, kidney, placenta, and peripheral blood leukocytes
<i>Tor4a</i>	Torsin-4a	Chaperone	No
<i>Frem1</i>	FRAS1-related extracellular matrix protein 1	cation transporter	No
<i>Dennd5b</i>	DENN domain-containing protein 5B	Ion channel, G-protein modulator, membrane-bound signaling molecule	Expressed in mature B cells
<i>Xaf1</i>	XIAP-associated factor 1		Expressed in T Helper cells

A*runx1***B***cmyb*

A

	Hb	WCC	PLT
LRP2 KO	13.9 (1.69)	7.4 (2.12)	1098.5 (49.39)
WT	15.1 (1.55)	7.1 (1.2)	1118.3 (200.3)

B



Supplemental Table S1

Genes Up-regulated in the FLK1+/LacZ- fraction

Gene symbol	LogFC	P-value
Edn1	1.087052505	0.544645462
Adam15	1.096967974	0.057858206
Klf2	1.552579775	0.043804917
Calcr1	1.65397083	0.009454124
Tnfaip2	1.678923375	0.007076656
Col18a1	1.680188937	0.000105422
Sh3tc1	1.68266225	0.239405654
Ptrf	1.686370299	0.020360381
Tmem204	1.698010214	0.364754734
Ddah1	1.701268811	0.002344438
Plxnc1	1.77132956	0.029150256
Ptprb	1.798755188	0.001642291
Hapln1	1.803827939	0.000180533
Flt4	1.81507194	7.60458E-05
Sox18	1.827185378	0.000253912
Parp14	1.830079852	0.003615943
Fmo1	1.880601627	0.662464724
Fli1	1.891716668	5.73862E-05
Arap3	1.934430362	0.000112014
Cbfa2t3	1.970349548	0.000457988
Jag2	2.028158816	0.01367377
Gap43	2.033613246	0.018886073
Erg	2.044460628	0.009601111
Oasl2	2.046144561	0.001696947
Car8	2.057906159	0.096011129
Gstm1	2.064067907	1.3604E-05
Plcg2	2.066293945	0.001221338
Rhoj	2.094338859	0.000155802
Glrx	2.119153348	0.001790813
Cbs	2.119967127	0.007751582
Myct1	2.120605065	0.108486122
Thbd	2.139519925	0.041800621
Lrp2	2.139519925	0.006199094
Pik3cb	2.142194059	0.001769992
Bcl6b	2.147425123	0.0003317
Gca	2.150555447	0.014679821
Dcaf12l1	2.16566234	0.045560884
Sox7	2.174728023	2.03373E-06

Gja4	2.188335841	0.021281851
Mageb16	2.201764164	0.028264699
Laptm5	2.205074479	0.00279795
Mtus1	2.260979437	0.001639892
Prrg4	2.287077113	0.0083592
Chchd10	2.292194746	3.08535E-06
Gcnt2	2.292862042	0.018055141
Tspan18	2.310009471	4.49457E-06
Ramp2	2.321386599	3.87678E-06
Lonrf3	2.333967245	0.080567576
Edil3	2.338014078	0.043433878
Tek	2.352213212	6.55371E-05
Rasip1	2.354911982	4.21616E-06
Lyl1	2.364162096	0.008989919
Slain1	2.367160424	0.016180347
Dysf	2.378863287	0.000146065
Spns2	2.454037875	0.000663005
Igfbp3	2.454515995	5.20963E-07
Gpm6a	2.465564128	0.372363712
Cd34	2.471393207	0.031684002
Icam2	2.472706197	0.000425494
Nefl	2.475228664	3.2853E-05
Smagp	2.530659156	0.022321854
Ushbp1	2.537713914	0.224722744
H19	2.53859547	2.89424E-10
C77370	2.53956471	0.002352499
Wipf3	2.59109501	0.000309686
Utf1	2.624496018	0.000790117
Hoxb5	2.6345403	0.04801989
Gfi1b	2.64244189	0.009106872
Ahr	2.658209206	0.037305532
Wscd1	2.678412777	0.029159008
Mt1	2.696325413	3.06058E-07
Thsd1	2.713341868	0.000693889
Pecam1	2.748445669	5.52696E-06
Gypc	2.775693652	0.000884256
Mir675	2.784317975	0.002039881
Mt2	2.797161886	4.91321E-08
Igf1	2.811136808	0.002047082
Cd93	2.83041587	0.001829152
Plek	2.852587251	0.0477939
Gad2	2.889245722	0.000556938
Ednrb	2.974577776	0.057961662
Abca1	3.002163607	0.07276324

Rin1	3.031910951	0.049704245
F2rl2	3.068905158	0.044838631
Esam	3.118761365	0.000276056
Mpo	3.240857841	0.026627781
Ldb2	3.248207297	0.009031311
Nos3	3.319713262	0.020452161
Fxyd5	3.350086911	0.008404157
Cdh5	3.523106166	3.47251E-12
Tie1	3.53897007	6.02652E-06
Oit3	3.672015006	0.015333772
Nr5a2	3.728598534	0.023161499
Trim47	3.734750761	0.087328428
Mfng	3.76512441	0.040224814
Disp2	3.812282387	2.30535E-05
Eld1	3.870658824	6.35113E-05
Ccdc85a	3.908082364	0.006066936
Clic5	4.087052505	0.014790403
Mecom	4.110899247	0.002374777
Ptchd1	4.954948969	0.009799116
Ltc4s	5.520011912	0.022456113
Gimap4	6.050526629	0.003415153
Zp3	6.153103569	2.73733E-05
Cldn5	6.224556029	0.001564233
C130074G19Rik	6.554975456	2.88197E-07
Tlr4	6.762764396	7.9993E-12

Supplemental Table S1

Genes Down-regulated in the FLK1+/LacZ- fraction

Gene symbol	LogFC	P-value
Omd	-3.443644727	1.43099E-07
Pbld1	-3.33667787	9.42527E-07
1500012F01Rik	-3.32524751	8.76912E-15
Cxcr2	-3.116079542	1.5724E-06
Zim1	-3.079224719	0.000273567
Rpph1	-3.035344126	0.000624533
Mpv17l	-3.029685259	4.94891E-06
Snhg6	-2.901632182	5.03045E-06
Gm16702	-2.881238635	1.63224E-05
Tmem86a	-2.83821662	0.037478878
Pcgf1	-2.766596069	0.000444155
Tyr	-2.707363361	0.017659225
Snhg1	-2.606178717	3.48776E-10
AA465934	-2.599303616	1.78453E-08
Tsacc	-2.534435872	0.015862459
Slc7a14	-2.475189919	0.000255652
Smim4	-2.459369534	0.00250001
Snhg12	-2.454883391	3.96865E-08
Mrpl33	-2.369692173	6.23265E-07
Nmrk1	-2.286351807	0.00020267
2810001G20Rik	-2.273010719	0.034753041
2500004C02Rik	-2.199542517	0.004688877
Rpl41	-2.195751742	1.59473E-08
Tnnc1	-2.191806868	0.009249219
Atp5l	-2.176233922	6.19553E-08
Phlda2	-2.174697829	1.18531E-07
AI450353	-2.170672062	3.44592E-05
2810008D09Rik	-2.166344208	0.001119544
Rab33b	-2.137786335	3.49429E-05
2410006H16Rik	-2.111274338	1.31921E-07
Gm11974	-2.104206638	1.12476E-05
1600020E01Rik	-2.074410918	0.011711608
Lepr	-2.067148144	0.029084436
1810032O08Rik	-2.059788883	2.83772E-05
Grb10	-2.045972914	9.4691E-07
H2-T23	-2.02743351	0.015336305
Polr2k	-1.943826808	0.000988815
Rps24	-1.912715704	7.20553E-07
Usmg5	-1.90756694	0.000121479

Chchd1	-1.861472579	0.000234435
Mnf1	-1.851159702	0.000475386
Tmem208	-1.846219535	0.000288931
Frem1	-1.835779635	0.029421539
4930412O13Rik	-1.832493391	0.000685628
Tnnt3	-1.831810732	0.014886289
Timm8b	-1.82503165	3.94814E-05
Rpl35	-1.81840748	3.01365E-06
Uqcrh	-1.803074561	1.23805E-05
Rpl13a	-1.794963422	3.84567E-06
1110059E24Rik	-1.793815299	0.000347094
2410004N09Rik	-1.754403645	0.001418213
Lsm3	-1.752929722	4.3554E-05
Acyp1	-1.752581652	0.003432837
Commd2	-1.749448763	0.001491238
Wdr38	-1.732902454	0.003299264
Ppp1r16b	-1.718392827	0.004212779
Ada	-1.716744598	0.049697011
Ndufa7	-1.712511296	7.87568E-05
N6amt1	-1.70718029	0.034158685
Ccdc107	-1.705505415	0.035432788
2810408A11Rik	-1.694307209	0.025011997
Snrpe	-1.685820743	6.01685E-05
Gnptg	-1.677097919	0.003908131
Cdkn1c	-1.671891458	0.004903075
Xaf1	-1.670731561	0.010160461
Taf1d	-1.653252435	0.000114362
Rps27l	-1.651472254	3.71671E-05
Rpl17	-1.617675036	2.56901E-05
Dnajc15	-1.605713285	0.004843374
Mrpl53	-1.605713285	0.002009382
A230046K03Rik	-1.589611808	0.000115813
Loxl2	-1.5752049	0.000796307
Trnau1ap	-1.574986219	0.005760675
Slfn5	-1.571567037	0.008555419
Cox7b	-1.558384248	0.00011155
Rps18	-1.544226652	5.6259E-05
Mfsd11	-1.543184076	0.002973958
Myeov2	-1.522099461	0.002501949
Ndufb8	-1.515810209	0.000503508
Ska1	-1.513220541	0.003933932
Znhit3	-1.495699452	0.002465377
Mrps35	-1.495365239	0.003322679
Tenm4	-1.493120394	0.013235397

Snrpg	-1.489592463	0.000147658
Far2	-1.48840291	0.01000884
Sec61g	-1.48254913	0.000229356
Churc1	-1.475573666	0.014071031
Dclre1c	-1.460417142	0.001742686
Pdlim3	-1.437809204	0.000747407
Pfdn5	-1.433673376	0.000465634
Hspe1	-1.358444684	0.000475662
Rpl36	-1.341678038	0.000419801
Gm15645	-1.34094818	0.007839282
Tmem69	-1.330831373	0.014474118
Casc4	-1.218342117	0.004189447
Tor4a	-1.19925168	0.034050492
Dennd5b	-1.165928236	0.02212856
Tmem206	-1.109755791	0.01054391
Cflar	-1.043723213	0.0278312
Lhx1	-1.027683631	0.016250115
Irak1	-0.151310873	0.82384325

Supplemental Table S2

Genes Up-regulated in the ENG-/LacZ+ HEI population

Gene Symbol	logFC	FDR
Trim33	-0.3573469	0.04650547
Gspt1	-0.3605518	0.04987142
Sqle	-0.3611902	0.04749712
Tardbp	-0.3666847	0.0450795
Tcerg1	-0.376228	0.04692825
Psemb6	-0.3846357	0.03435802
Hnrnph1	-0.3850724	0.04138392
Orc3	-0.386385	0.04950145
Sf3a1	-0.3926086	0.02979202
Med14	-0.3953839	0.03534769
Ola1	-0.396888	0.03082871
Stip1	-0.3980127	0.02573146
Rrm1	-0.40015	0.04897333
Set	-0.4020976	0.01963419
Pdcd11	-0.4036084	0.03644631
Pkm	-0.4037588	0.03312645
Ncapd2	-0.4068065	0.03463265
Xpo5	-0.4114309	0.04382674
Utp6	-0.4115701	0.03127257
Wdr75	-0.4123941	0.03276747
Tyms	-0.4180273	0.03074998
Mybbp1a	-0.4199431	0.02769257
Smarcc1	-0.4207404	0.01059255
U2af2	-0.4218187	0.03635639
Nup62	-0.4218858	0.03659348
Epb4.115	-0.4222133	0.03493345
Ddx18	-0.4238809	0.01903771
Cops5	-0.4239614	0.04751996
Znrf3	-0.4252158	0.03912092
Cirh1a	-0.4273012	0.02573146
Plagl2	-0.4287221	0.03711419
Tll4	-0.4302274	0.03645358
Rae1	-0.4322049	0.02672746
Fam210a	-0.4326129	0.03337002
Larp7	-0.4335868	0.01353108
Hnrnpc	-0.433706	0.04463755
Cpsf6	-0.4344144	0.0310876
Nsun2	-0.4344325	0.0494033
Nme1	-0.4416846	0.0185303

Aifm1	-0.4417268	0.03947684
Denr	-0.4420595	0.04877194
Mre11a	-0.4431858	0.03997574
Nup160	-0.445214	0.01700972
Nup155	-0.4457544	0.01493718
Psemb3	-0.4494857	0.02443424
Snrnp70	-0.4500929	0.0349515
Smn1	-0.4510802	0.04776154
Fasn	-0.4512933	0.01263781
Plcl2	-0.4526148	0.0450795
Fastkd2	-0.4526201	0.04305208
Dtl	-0.4527959	0.0459797
Gins2	-0.4540335	0.04249431
Ppat	-0.4559212	0.01150381
Fus	-0.4564607	0.0163981
Dhfr	-0.4572799	0.0368505
Atic	-0.457867	0.03899655
Acaca	-0.458071	0.03301159
Mms221	-0.458251	0.04369371
Wdr3	-0.4585454	0.04762262
Utp11l	-0.4591145	0.04692825
Hat1	-0.459847	0.03971781
Usp24	-0.4598783	0.01399932
Hells	-0.4618291	0.019913
Rsl1d1	-0.4630114	0.00897587
Tacc3	-0.4632733	0.02304744
C1qbp	-0.4654797	0.01928416
Khsrp	-0.4659537	0.02543228
Erh	-0.4663217	0.01225007
Nudc	-0.4675083	0.02315338
Eif4e	-0.4700993	0.01482001
Ruvbl2	-0.4714274	0.01715585
Frs2	-0.4737451	0.02003154
Fgfr1op	-0.4764688	0.02174393
Rsl24d1	-0.4794557	0.03088623
Gatad1	-0.4821751	0.00481321
Phb	-0.4823853	0.026337
Park7	-0.4844912	0.03428394
Tsr1	-0.4870355	0.02543228
Chaf1b	-0.4875325	0.03194556
Myo19	-0.4878522	0.04125506
Trmt6	-0.4884361	0.0349515
Cct3	-0.4887553	0.00537465
Chek1	-0.4912268	0.03306718

Rcc2	-0.4921252	0.01422541
Cacybp	-0.4933062	0.0278088
0610007P14Rik	-0.4934913	0.03218175
Ticrr	-0.4936432	0.01544719
Oat	-0.494166	0.00510136
Ing5	-0.4949474	0.02626481
Psmg1	-0.4960053	0.01868596
Tuba1c	-0.4960215	0.00190905
Cks1b	-0.4983408	0.01006836
Dkc1	-0.500082	0.0255021
Mlec	-0.5010375	0.00379324
Wdr18	-0.5016548	0.02760258
Eef1d	-0.5016561	0.00502959
Gpn1	-0.50166	0.03127257
Kif20a	-0.5021924	0.02025168
Gfpt2	-0.5025914	0.03889119
C330027C09Rik	-0.503346	0.0207671
Rrp1b	-0.5039365	0.0041559
Jade3	-0.5045377	0.04865351
Phlda1	-0.511139	0.01548462
Mrps30	-0.5112446	0.03023915
Nsdhl	-0.5131118	0.00403504
Emg1	-0.514678	0.03023915
Pitrm1	-0.5148078	0.03672314
Dazap1	-0.5151004	0.04344302
Pcna	-0.5155391	0.00610925
Eno1b	-0.5159822	0.02790907
Pspc1	-0.5165724	0.02684948
Cenph	-0.5175012	0.03473194
Srm	-0.5186396	0.01289603
Uhrf1	-0.5191902	0.00229883
Nol10	-0.5216314	0.03622576
Eif5a	-0.5225901	0.00090198
Rfc5	-0.5234656	0.04012009
Seh1l	-0.523609	0.00115992
Mycbp	-0.5236966	0.023239
Sf3b4	-0.5257505	0.01150381
Gemin5	-0.5259505	0.01236071
Pno1	-0.5261394	0.00501727
Mrpl18	-0.5270604	0.00714937
Hspd1	-0.5276276	0.0162064
Phykpl	-0.5278217	0.01967461
Nop58	-0.5278991	0.00264092
Hsp90aa1	-0.5285458	0.00455905

Klh21	-0.5298036	0.02020045
Rspo3	-0.5305875	0.00367767
Magoh	-0.5325781	0.0217567
Pfklp	-0.5343331	0.03711419
Casc5	-0.5346706	0.01063502
Katna1	-0.5392394	0.04053541
Haus1	-0.5416084	0.0184151
Ddx20	-0.5419028	0.00725007
Zwilch	-0.5423232	0.0035169
D8Erttd738e	-0.5444438	0.0362936
Ppal	-0.545403	0.04828985
Isoc1	-0.5481145	0.0163981
Lin28a	-0.5484315	0.00615527
Cse1l	-0.5488957	0.00026741
Lss	-0.5497879	0.01150381
Mad2l1	-0.5499787	0.01318802
E2f4	-0.5500669	0.01668018
Cep76	-0.5505935	0.03034652
Noc3l	-0.5510514	0.00893186
Rap1b	-0.5510983	0.02978328
Ddx21	-0.551379	0.00206249
Zfp850	-0.5528011	0.03489856
Mrps22	-0.5561946	0.0331987
Ranbp1	-0.5567163	0.0046022
Umps	-0.5584059	0.0069547
Dut	-0.5585744	0.00184012
Mcm7	-0.5601798	0.0006588
Cep290	-0.5611988	0.0289515
Mif	-0.5644379	0.02222295
Usp36	-0.5669756	0.02926058
Ppp1r14b	-0.5692128	0.00604927
Hdgf	-0.5709078	0.00033317
Hist1h1e	-0.5717303	0.01421933
Cluh	-0.5718228	0.01021717
Cks2	-0.5722872	0.03797658
Gesh	-0.5728595	0.00190905
Ahsa1	-0.574962	0.0013853
Pold1	-0.5749853	0.01981412
Sms	-0.5751655	0.00384989
Jmjd6	-0.5756012	0.00729657
Fbl	-0.5758456	0.00085362
Prps1	-0.5765977	0.04076364
Nop56	-0.5766727	0.03773238
Homer1	-0.5783565	0.03549961

Nhp2	-0.582946	0.00138054
Nasp	-0.5832083	0.0001497
Noc2l	-0.5835108	0.00115149
Rcc1	-0.5838483	0.01150381
Atad5	-0.5839931	0.00074499
Snrpd2	-0.5845768	0.04959674
Gsg2	-0.5887483	0.02480468
Mdk	-0.5925585	0.03677938
Maz	-0.5930999	0.03107751
Mrpl3	-0.5936589	0.01062553
Naf1	-0.5942042	0.00565761
Mid1	-0.5945538	0.01015109
Cenpn	-0.5953636	0.00738044
Pprc1	-0.595594	0.01073499
Dis3	-0.5961683	0.00051683
Naa10	-0.5967393	0.01483212
Ankrd27	-0.5970243	0.03643087
Rpp14	-0.5981019	0.03203946
Gnl3	-0.6009705	0.00426017
Pa2g4	-0.6011336	7.3392E-05
Kmt2b	-0.60144	0.03572526
Ddx39	-0.6014412	0.00136878
Nop10	-0.60184	0.00626164
Snape4	-0.6024253	0.00972513
Sppl2a	-0.6039032	0.01632944
Bop1	-0.6056497	0.00679395
Psat1	-0.6060635	0.00091311
E2f1	-0.6070543	0.01963896
Cap2	-0.6076249	0.00050595
Polr1b	-0.6098149	0.00253093
Naa50	-0.6103831	0.02790907
Nup35	-0.6118891	0.03855556
Timeless	-0.6145758	0.00215836
Pfas	-0.616097	0.00089948
Rps12	-0.6173728	0.03182126
Aste1	-0.618063	0.03142146
Zmym1	-0.6181706	0.01059225
Dtymk	-0.6182236	0.02642009
Kif15	-0.6183403	0.00027944
Haus3	-0.6188925	0.00930685
Mrpl52	-0.6198458	0.04870071
P2ry1	-0.6275068	0.02693819
Dusp18	-0.628064	0.0128828
Ccdc124	-0.6290632	0.02040009

Ell2	-0.6296718	0.01841746
Tk1	-0.6301929	0.03728596
Shmt2	-0.630889	7.9875E-05
Abcb10	-0.6330012	0.00130076
D030056L22Rik	-0.6356704	0.01811323
Akt2	-0.6369274	0.02134294
L2hgdh	-0.6389627	0.00550733
Rreb1	-0.6400927	9.9507E-05
Elac2	-0.6413754	0.00215836
Etl4	-0.6415104	0.00152996
2410002F23Rik	-0.6417525	0.01963896
Nkrf	-0.6418171	0.00353932
Mrpl47	-0.6420686	0.03473194
Lsm2	-0.6427002	0.01253675
9430015G10Rik	-0.6469001	0.02885122
Uchl3	-0.6487112	0.0089163
Dhodh	-0.6498502	0.02416471
Pole	-0.6524743	0.00118802
Nmral1	-0.653271	0.04645893
Rad51	-0.653761	0.00406655
Urb1	-0.6553645	0.00627884
Mob1b	-0.6574739	0.00245262
Nolc1	-0.6576932	8.6466E-06
Bckdhh	-0.6580894	0.02211293
Bag2	-0.6645741	0.02505003
Smyd5	-0.6656635	0.02672146
Ndufs6	-0.6699754	0.01536491
Itga2b	-0.6727368	0.02027658
Tsen2	-0.6775487	0.04646687
Edrf1	-0.6829337	0.00804993
Ppan	-0.6850071	0.03564443
Ptgr1	-0.6889211	0.02285697
Cdc6	-0.6929623	0.00174087
3110082I17Rik	-0.6937728	0.01223807
Diablo	-0.6948298	0.02997505
Rybp	-0.6951969	0.04477566
Dctd	-0.7007772	0.0025433
Mob3c	-0.7061391	0.0292513
Ippk	-0.7063486	0.01195282
Rab11fip1	-0.7064745	0.00787161
Mrpl22	-0.7076716	0.01843504
Sod2	-0.7101316	0.00083047
Srxn1	-0.7117592	0.02027658
Tlcd1	-0.7161115	0.0349515

Mtrr	-0.7165168	0.00942764
Uqcr10	-0.7168167	0.00711417
Heatr2	-0.7168978	0.00627968
Fancd2	-0.7181052	0.0012332
Tcof1	-0.718714	0.00017748
Trip13	-0.7194061	0.000102
Rad54l	-0.7201007	0.00644358
Rpp40	-0.720515	0.00872237
Tal1	-0.7243217	0.03500468
Ccdc18	-0.7250853	0.02243067
Zfp36l2	-0.7253139	0.00185707
Setd8	-0.7259416	0.00026996
Prkab1	-0.729529	0.00012967
Zbtb43	-0.7306579	0.03843164
Gins1	-0.7321125	0.00943833
Sf3b6	-0.7375405	0.02223724
Cep152	-0.7386925	0.03594749
Ung	-0.7392586	0.01686781
Snrpa1	-0.741571	0.00020826
Slc16a6	-0.7451852	0.01005037
Ska3	-0.746177	0.00597189
Egln3	-0.7466605	4.2368E-05
Bcor	-0.7480644	1.9447E-06
Zik1	-0.7482625	0.02993161
Srf	-0.7496534	0.00348084
Snhg5	-0.7520657	0.00095124
Uqcr11	-0.7552484	0.01150381
Ddx11	-0.7563069	0.00417648
Psmc3ip	-0.7568939	0.00041982
1110038B12Rik	-0.7581591	0.00127361
Helb	-0.7590714	0.02052056
Hsd17b7	-0.7599393	0.00067447
Prkcd	-0.7609372	0.04692825
Smim3	-0.7616062	0.01132173
Notch2	-0.7648085	0.00160718
Hmgn2	-0.7648535	0.00365518
Lsm5	-0.7653828	0.02076879
Tfb2m	-0.7675262	0.016153
Anapc11	-0.7680224	0.03422569
Ppih	-0.7682877	0.01636778
H2afx	-0.7685989	0.00059415
Fancb	-0.7713014	0.04139186
Gyk	-0.7715793	0.0468364
Polr2i	-0.7720022	0.01984889

Depdc1b	-0.7720366	0.00170735
Zcchc10	-0.7745844	0.00524108
Snrpg	-0.7818499	0.04215328
Klhl12	-0.7832182	0.00181501
E2f8	-0.78417	0.00018093
Epb4.1	-0.7849334	1.3779E-05
Wbscr16	-0.79124	0.01821397
Fth1	-0.7925434	0.00031048
Troap	-0.7972141	0.0231519
Hspe1	-0.7988842	0.01963896
Rnd2	-0.8016923	0.02263465
Ern1	-0.8027457	0.0042741
Mtx3	-0.8052675	0.04224171
Suv39h2	-0.8053629	0.00026429
Tcf7	-0.8056699	3.7094E-05
Lyar	-0.806388	5.6751E-06
Smtnl2	-0.8080904	0.0132248
Tnfaip8	-0.8116157	0.00189608
Peo1	-0.8136152	0.00721895
Speg	-0.81617	0.0217567
Fut8	-0.8166749	0.00010455
D2Wsu81e	-0.8168487	0.00815552
H2afz	-0.8234957	3.0782E-08
Laptm5	-0.8267113	0.04336512
Ubxn2a	-0.8271654	0.00022276
Stra13	-0.8316425	0.01229853
Rrp12	-0.8351638	0.00010987
Gcnt1	-0.8393189	2.3531E-05
Tsc1	-0.8417155	0.00012396
Trmt61a	-0.8425698	0.00089333
Polr2f	-0.8473131	0.00719128
Utp23	-0.8561307	0.01021231
Mrto4	-0.8586158	0.00010066
Fads2	-0.8661943	4.8134E-06
Tm7sf2	-0.8688088	0.02543228
Nbeal2	-0.8716841	0.00662327
Skal	-0.8730391	0.03653303
Traip	-0.8757748	0.02069251
Psph	-0.8766564	0.00684254
Inip	-0.8770961	0.00171834
Ormdl1	-0.8771678	0.00814927
Srl	-0.8787499	0.02313439
C530008M17Rik	-0.8817309	0.02435718
Hist1h1b	-0.883334	5.4691E-05

Caprin2	-0.8843534	0.00565763
RbmX	-0.8861848	0.00045963
Grap2	-0.8868077	0.0243308
Sssca1	-0.8898676	0.00050595
Gas5	-0.8931038	0.00100565
Melk	-0.8938092	0.00074806
Idi1	-0.8942903	0.00062584
Bcat1	-0.9044063	0.00614126
Mdm1	-0.9053117	7.9056E-05
Rps6ka6	-0.9072983	2.512E-06
Prmt1	-0.9141192	0.00027692
Fkbp11	-0.9174265	0.03022019
Slc19a1	-0.9234819	0.01292404
Ppil3	-0.9241589	0.03307018
Gtf3c5	-0.9250698	8.0921E-08
Hist1h2bb	-0.9256411	0.03473194
Spc24	-0.9296335	9.0055E-05
Itga4	-0.9299064	0.00012351
Hist1h2be	-0.9304375	0.02222295
Pola2	-0.9380601	1.6172E-05
Ahi1	-0.9508248	2.5154E-05
Acy1	-0.9528935	0.04380506
Ctla2a	-0.9543965	0.04970076
Cenpp	-0.9595026	0.01722529
Gpc6	-0.9623014	0.01029804
Shroom3	-0.9652569	0.03815161
Zfp692	-0.9781341	0.00385429
Tmem158	-0.9823048	0.02592136
Cited2	-0.9926737	1.7574E-06
Taf1d	-0.9953624	0.03696605
Acot11	-1.0008928	0.00777375
Lsm7	-1.0009126	0.00430527
Slc7a5	-1.0036	2.178E-06
Tmem86b	-1.0039618	0.01746706
Ifi30	-1.0044766	0.04870071
Psd3	-1.0174544	0.03534982
Wdsub1	-1.0239369	0.04895047
1810032O08Rik	-1.0328231	0.02993161
Hist1h4d	-1.0383794	0.00550733
Dctpp1	-1.0393153	0.00031738
Plscr1	-1.039389	0.00461492
Pmp22	-1.0535001	0.00984758
Mgat4a	-1.0568436	0.00777681
C77080	-1.0585692	0.00018931

Snhg3	-1.0648738	0.01026912
Sec61g	-1.0666418	0.01710869
Psmg4	-1.0676331	0.04632492
Sdc1	-1.0753188	0.00099975
Fzd2	-1.0778418	0.02796823
Lhpp	-1.0879831	0.02047849
Gse1	-1.0939363	2.3134E-11
Zfp930	-1.0940181	0.0006457
Lsm3	-1.0963022	0.00452377
Tagln	-1.1030145	0.0347611
Car2	-1.1035294	0.03598601
Kcnj5	-1.1045913	0.00054441
Med30	-1.1093461	0.01936224
Dsp	-1.1110028	0.00991782
Lpar1	-1.1156382	0.0338615
Kalrn	-1.1171644	1.0778E-06
Ccdc134	-1.1231804	0.02068478
Xk	-1.1366802	0.00784992
Dyrk2	-1.1426776	0.00974385
Irs1	-1.1430887	0.00296595
Kctd15	-1.1434019	0.02817557
Lyl1	-1.1442502	0.00095854
Apitd1	-1.1442509	0.00232128
Ank3	-1.1478545	5.4748E-07
Etv2	-1.1482494	0.00335705
Arhgap6	-1.1576452	1.2625E-05
Tpm2	-1.1599192	0.00522762
Cth	-1.1612232	0.03283644
Mtfp1	-1.162728	0.01029804
Chchd1	-1.1640701	0.04268792
Fam109b	-1.1687442	0.03442235
Snrnp25	-1.1795927	8.6466E-06
Tfrc	-1.1817508	1.4296E-15
Fzd10	-1.1850427	0.01202788
Robo2	-1.1976152	0.02796823
Rab27b	-1.2026754	0.00026257
Hist1h3e	-1.207497	0.00202841
Hist1h2bm	-1.2087727	0.00016605
Pde4dip	-1.2117548	2.2216E-08
Myl9	-1.2154017	0.00896662
Col5a1	-1.2365169	0.02412473
Nfix	-1.2439774	0.02885122
Plek	-1.2460721	0.02967711
Hist1h2ac	-1.2654033	0.04800539

Sh3bp1	-1.2655429	3.8227E-06
Slc35f2	-1.2714037	0.00447928
Med12l	-1.2776314	5.1885E-08
Eno3	-1.2819185	0.00053602
Gria2	-1.2929235	0.01273978
Prtg	-1.2975163	3.8001E-10
Samd14	-1.3126027	0.03439808
Gfra2	-1.3188517	0.00121213
Hist2h2ac	-1.3249293	0.04380506
Hist1h2bl	-1.3323982	3.9428E-06
Hist1h4c	-1.3424522	0.03677938
Lgals9	-1.3443204	0.00115378
Phgdh	-1.3465429	1.0238E-06
Hist1h4a	-1.3568512	0.04092191
Hspb8	-1.3581106	0.04765362
Hist1h2bf	-1.3608963	9.8475E-05
Lama1	-1.3748046	0.01053971
Ptpre	-1.3799639	6.3333E-07
Mpl	-1.383543	0.00145656
Gfi1	-1.3873919	0.0297813
Tenm3	-1.3946725	0.03673257
Klf8	-1.398728	1.0931E-09
Cyp26b1	-1.4037073	0.0103534
Hist1h2ak	-1.4072807	0.00027529
Pitx2	-1.410157	0.00313612
Dyrk3	-1.4106397	0.00457984
Nap1l2	-1.4284217	0.04125506
Ston2	-1.4291669	0.00010666
Hist1h3g	-1.4334317	0.00190905
Ifitm1	-1.4344149	5.8211E-05
Adamts9	-1.4390785	0.02008021
Mrv1	-1.439407	0.0163981
Sfrp2	-1.4502113	0.03473194
Lrr1	-1.45511	0.00446531
Mpo	-1.4663655	0.01179793
Ryr2	-1.4690604	0.016935
Unc5c	-1.4771939	4.1464E-15
Egfl8	-1.4885643	0.04839119
Fam117a	-1.4937007	0.00425314
Hist1h2bh	-1.4980855	4.3762E-05
Syt7	-1.5153341	0.01778145
Gpc3	-1.5323008	7.1689E-09
Sall3	-1.5370094	1.5078E-17
Wnt5a	-1.5468432	0.0007608

Hist1h3i	-1.5483412	0.02672146
Plcxdl	-1.5523584	0.0381536
Hist1h2bp	-1.5681605	0.00989979
Hist1h2bk	-1.5735921	0.00043405
Snord99	-1.5909402	0.01629015
AI467606	-1.5969964	0.02294639
Ptprj	-1.6077737	1.0931E-09
Ctse	-1.6078993	0.00243166
Prkca	-1.6098811	3.3616E-10
Rgs10	-1.6222642	0.01325467
Wisp1	-1.63618	0.00067447
Hist1h1a	-1.6408645	0.00010791
Dlg3	-1.6444894	0.03846739
Krt19	-1.6450808	0.00471472
Pappa	-1.6741823	0.02022074
Cnn1	-1.6778678	0.0287664
Dapp1	-1.7240331	1.2642E-07
Ccnd2	-1.7249129	8.4849E-05
Sdc2	-1.7252224	0.00129517
Slc30a3	-1.7763864	0.02078334
Hist1h2ai	-1.8043194	0.04861388
Nrgn	-1.8055743	0.0099646
Aif1l	-1.8075623	0.00011715
Atp2a3	-1.8125327	2.5755E-06
Rps6kl1	-1.8244938	0.0494033
Gm16982	-1.848031	0.03181333
Tmem62	-1.8548086	0.01330257
Thsd4	-1.867671	0.00223766
Colla2	-1.8752501	0.00086706
Kcnj2	-1.8778166	0.00938174
Itgb3	-1.904678	1.0396E-06
Snord47	-1.908005	0.03506548
Hist1h4k	-1.9096921	0.00594326
Gng7	-1.9207414	0.01967723
Ackr3	-1.9236598	0.00111278
Fam46a	-1.9265152	0.00730926
Cdh11	-1.9347074	0.00368123
Acta2	-1.9431264	0.00108295
Igsf11	-1.9730923	0.0032421
Hbb-y	-1.9748359	4.584E-05
Ubxn11	-1.983615	0.00684254
Ikzf2	-1.9955979	8.666E-16
Epcam	-1.9977628	0.01015501
Was	-1.9990616	8.5009E-05

Acat3	-2.0060747	0.02734464
Mcoln3	-2.0135873	0.03269587
Hist1h4j	-2.0168569	0.03711419
Gli2	-2.0256238	0.0017032
Smyd1	-2.0480579	0.01838017
Pde11a	-2.0583309	0.02803373
Map7d2	-2.0666869	0.03246469
Wwc1	-2.0764389	0.00047185
Hba-x	-2.0906682	3.159E-07
Irs2	-2.0961118	5.8778E-20
Pdgrb	-2.1019287	0.00743475
Ptpn13	-2.1059753	4.7899E-08
Nfe2	-2.1065983	4.4655E-08
Smim1	-2.1151748	0.01490303
Fgf10	-2.1307131	1.5168E-05
Wnt4	-2.1362897	0.00140192
Snord15b	-2.157675	0.00934557
Syt15	-2.1590435	0.00044028
Syne4	-2.1602438	0.00603873
Lrp2	-2.1683193	0.00012169
Kcnd3	-2.1909069	0.00690389
Kif26b	-2.2020228	0.03473194
Tspan32	-2.2086198	8.0245E-06
Gfi1b	-2.2107003	1.6969E-06
Cgn	-2.2139564	0.00221779
Chpf	-2.2346358	0.02143951
Col12a1	-2.2377922	1.3079E-05
Mir1949	-2.252971	0.0065173
Reep6	-2.2591005	0.00044188
Gucy1a3	-2.2596713	3.0558E-09
Fam110c	-2.2660124	0.04749712
Pkd113	-2.2721338	0.02464859
Col8a1	-2.275621	0.00458619
Ikzf1	-2.2786084	1.5466E-16
Phlda2	-2.2808251	0.00054441
Sdk2	-2.3005852	0.00178985
Cort	-2.3033757	0.04319604
F3	-2.3463844	0.00249194
Ildr2	-2.3554778	0.02541073
Cnksr2	-2.4145604	0.00174087
Adamts3	-2.4415041	1.1435E-16
Slit3	-2.4443195	0.01676172
Ccl17	-2.4618337	0.00565698
Scube2	-2.4633338	1.2498E-05

P2rx1	-2.4648688	4.2409E-06
Plbd1	-2.4842592	0.04380506
Nog	-2.4867721	0.03074998
Evx1	-2.5070756	0.00710432
Pard6b	-2.5132174	7.0216E-11
Dlk1	-2.5174664	0.01117957
Lypd6	-2.527006	0.01207909
Slc16a10	-2.5476922	1.0481E-05
Igfbp2	-2.5554076	1.8526E-05
Epb4.2	-2.5598202	1.8021E-05
Krt8	-2.5741038	1.0158E-06
Terc	-2.5834174	0.01929542
Hoxd1	-2.621979	8.1501E-07
Myl4	-2.630741	0.00770151
5330426P16Rik	-2.6446846	0.02899933
Adamts15	-2.6619076	0.00183546
Klhl34	-2.6888257	0.0243308
Snord65	-2.6966047	0.01369938
Zic3	-2.7075066	0.01007745
Chac1	-2.7158122	0.03425189
Car12	-2.7161838	0.0125579
Runx1	-2.7256084	1.6576E-27
Gpr56	-2.7306544	6.1352E-12
Lypd1	-2.7503944	0.02958715
Bdnf	-2.7569741	0.01706797
Cemip	-2.7597959	0.00972513
Psd2	-2.7685594	0.04023191
Bhlhe22	-2.7788676	0.04589984
Nkx1-2	-2.8116603	4.8282E-06
Krt18	-2.8300695	1.9496E-05
Fgf15	-2.8448297	0.00074499
Ripk4	-2.8502852	0.03564443
Acta1	-2.8507773	0.00670684
Mfsd2b	-2.8581726	3.3044E-10
Hbb-bh1	-2.8585705	2.1373E-10
Nnat	-2.870562	0.00943661
1700024P16Rik	-2.9094172	0.04618593
Trem12	-2.9188545	0.01644824
Il18rap	-2.9367721	0.02558671
Cdh22	-2.9609251	0.04793262
Lgr5	-2.9755872	4.1171E-26
Rgs4	-3.045216	0.00427405
Bmp8a	-3.0462458	0.00051332
Enpp1	-3.0546272	4.1166E-06

Asic4	-3.0661955	0.00893186
Coll1a2	-3.1009018	0.02666483
Igfbp5	-3.1027981	5.0016E-11
Scube3	-3.1383901	0.00062887
Crabp2	-3.2575996	0.00615527
Kcnk1	-3.2686447	3.722E-05
Rpph1	-3.281995	2.1394E-05
Lmod1	-3.2934985	0.02731732
Cacna1d	-3.3339941	0.01548462
Dpf3	-3.3366168	0.00736531
Gm14204	-3.3480536	0.00322228
Cdh3	-3.3515719	4.8587E-06
Svep1	-3.3665107	0.0246003
Plac8	-3.3850148	0.03516285
Ppp1r14a	-3.3875344	0.02733033
Snord11	-3.4033326	0.03447524
Col2a1	-3.4043366	0.00770291
Krtap5-4	-3.4274958	0.01385011
Tenm4	-3.4333517	0.00039229
Postn	-3.4429613	8.2311E-10
Myb	-3.4543925	8.3365E-09
Inhba	-3.4548191	0.00071399
Tgfb3	-3.4614945	0.00489641
Slc32a1	-3.4787561	0.01146119
Twist2	-3.486109	0.01341197
Vgll3	-3.5369871	4.2262E-07
Actc1	-3.5579124	7.5035E-08
Bnc2	-3.5880639	0.00947273
Reln	-3.6170028	3.5E-25
Krt14	-3.6405079	0.03969407
Sycp2	-3.6541894	0.03473194
Olfir893	-3.6656714	0.04406622
Colla1	-3.6976688	4.9544E-05
Slc7a8	-3.7032242	5.5358E-19
Snord49b	-3.7060819	0.01634423
5830432E09Rik	-3.7217186	0.0184344
Mir3473	-3.7421314	0.04317957
1700019A02Rik	-3.8088791	0.01171316
Lox11	-3.8129879	0.00049731
Mfap4	-3.8436132	1.6274E-06
Slc14a1	-3.9021836	0.03207504
Ms4a4d	-3.9106388	0.00987801
Arpp21	-3.9845324	0.01348456
Tuba8	-4.0014776	0.00446531

B4galnt3	-4.0405548	0.0170189
Mir1188	-4.0457792	0.04714171
Cbln1	-4.0779217	1.7455E-06
Bzrap1	-4.0941697	0.02551107
Mycbpap	-4.1183436	0.01331954
Tacstd2	-4.1381791	0.0168138
Mir6516	-4.1802058	0.00018038
Nrip3	-4.2215567	6.594E-12
Pdzk1ip1	-4.2568504	4.6365E-06
Ajap1	-4.2816439	0.04380506
Ccl3	-4.4113143	0.00739012
Muc13	-4.4403089	4.5577E-05
Cobl	-4.4440277	0.01229853
Dlx5	-4.4620843	0.0226765
Col8a2	-4.5076296	0.02801605
D830026I12Rik	-4.542468	0.00273577
Smoc2	-4.5827836	0.04994031
Mir6940	-4.5833125	0.02009453
Sema5b	-4.5908909	0.00018517
Ndufa4l2	-4.6090649	0.00112525
Slc1a6	-4.6456547	0.00116
Pgm5	-4.6485806	0.02672146
Gabra4	-4.7118276	0.04261054
Alx3	-4.8471877	0.01150381
A4gnt	-5.1071371	0.03788653
Vat1l	-5.1080698	0.0032421
Slitrk2	-5.1635952	0.0041559
Pip5k1l	-5.1675678	0.01401483
Mir1940	-5.2146295	0.03653303
Dppa5a	-5.2384808	2.2469E-06
Tph1	-5.285813	0.01755235
Bmp7	-5.3633196	0.00053602
Atp6v0d2	-5.5623301	0.000386
Pitx1	-5.5980066	0.01330257
Olfml1	-5.6903819	0.00127361
Prr15l	-5.7339197	0.01788615
BC027072	-5.7931802	0.01183079
Zfp641	-5.8463841	0.04897333
Gata1	-5.9307033	1.1428E-08
Fgf3	-5.9991188	1.1575E-07
Rnase6	-6.0909008	0.04215328
Lum	-6.1112301	0.0207671
Vmn2r2	-6.1502481	0.02629604
Atp6v0a4	-6.270693	0.0248442

Clec18a	-6.5983151	3.5931E-05
Elfn2	-6.6897525	0.00171301
Cd164l2	-7.1662694	0.00307347
Wfdc17	-7.1680774	0.00025308
Fstl3	-7.2822667	0.00190905
Tmem54	-7.5876681	0.00015351
Orm1	-7.628743	0.00086706
Sema4f	-8.0860262	0.00217464

Supplemental Table S2

Genes Down-regulated in the ENG-/LacZ+ HEI population

Gene Symbol	logFC	FDR
Crybb3	6.25650192	0.000354722
Cxcl17	6.15729044	0.006121384
Ly6g6e	6.14113513	0.015207308
Clcf1	6.12886931	0.000344337
Ccdc87	6.01920364	0.002263224
Kcnma1	5.81748514	0.00052578
Gja5	5.73413275	4.32555E-09
Slc41a2	5.54592025	6.60249E-06
Pcdhb14	5.41579617	0.005325232
Cftr	5.33818775	0.006788754
Hc	5.20317026	0.02040009
Fbxo40	5.13542033	0.014225415
Adamts15	5.10235581	0.01616849
Nlrp1b	5.04492195	0.015260874
Il7	4.93619933	7.33334E-05
Bmx	4.91850392	0.000121367
B230119M05Rik	4.85139145	0.019634188
Tbx3os2	4.78746119	0.046854014
Yipf7	4.78451146	0.015219914
Gbp11	4.78136599	0.020574987
Ido1	4.76348461	0.041332448
Eva1a	4.76262161	0.000251395
D930020B18Rik	4.75115005	0.03177312
E230025N22Rik	4.74596006	0.023740855
Tmem82	4.73257398	0.048941292
Fam19a3	4.69408279	0.000136769
Snph	4.57833549	0.041747333
Fam19a2	4.51858612	0.04735918
Trpm3	4.50268817	0.032075036
C1qtnf2	4.39069707	0.009430587
Clvs1	4.38407592	0.005507327
Gpihbp1	4.36945782	0.005771646
Pcdh9	4.35360008	1.47967E-09
S1pr4	4.21985657	0.00743091
Ifit1	4.18072192	0.01150381
Ccbe1	4.09615522	0.006109254
Ablim3	4.02702263	0.00216256
C1qtnf1	3.97657648	5.84737E-06
Abcd2	3.70273365	0.000520706

Npvf	3.70199549	0.029404986
Trnp1	3.6755603	0.024192528
Dpp4	3.66117546	0.008422956
9030617O03Rik	3.63402294	0.010635018
Ifit3	3.63391696	0.04613748
Scn3a	3.62772869	0.000267407
Sct	3.62674208	0.022430669
Slc2a13	3.57917944	4.57639E-07
Pdlim3	3.57766279	2.16822E-44
Cyp1a1	3.57720408	0.001905685
S100a16	3.5477518	2.13634E-11
Ly6a	3.42207613	2.62151E-12
Wbscr17	3.39468348	0.000355265
Pgbd5	3.38836145	1.56978E-05
Il1r1	3.34347429	6.40325E-07
Nr5a2	3.29320254	0.005750421
Cabp1	3.2728878	0.001120493
Grik3	3.26096666	0.018407656
Grin2c	3.24189718	0.005374654
Tmem53	3.22142009	0.034324076
Ramp3	3.20739422	0.048766901
Fst	3.19328629	2.98063E-08
Frrs11	3.12797418	0.000686358
Tmem150c	3.12351529	0.037114187
Art3	3.08782442	0.012536686
Disp2	3.08746265	0.000118107
Rgs17	3.0588474	7.52981E-05
Clec1a	3.05635354	6.05317E-14
Gbp6	3.03879822	0.022181358
Slc4a8	3.02118734	0.001524407
D430019H16Rik	3.01525558	0.000230621
Dusp26	2.9637332	0.01634546
Prnd	2.92446708	0.013206311
2700070H01Rik	2.88477997	0.032301302
Arhgap22	2.8796337	0.048204754
Pdlim4	2.85391918	0.00816996
Sorcs1	2.85196135	0.001475148
Col13a1	2.84329058	0.019212038
Adrb1	2.8354098	0.000456481
Add2	2.80979515	2.00059E-07
Bcl11b	2.80191944	0.012637806
Tifa	2.77963433	0.008198429
Lsamp	2.77214612	0.023419121
Robo4	2.76257106	9.41069E-17

Pth1r	2.76119957	0.001401918
Cyp2j9	2.75723043	0.032898869
Thrb	2.74835614	0.028183712
Mamld1	2.72417862	3.71113E-10
Gabrb2	2.68033058	2.17158E-05
Tmem51	2.67281111	0.019634188
Pcdh20	2.66722287	0.001611227
Bean1	2.65069942	0.012145624
Ceacam1	2.6373882	1.44202E-05
Sema3g	2.61765129	0.043021926
Gpr85	2.59638848	0.03182126
Pde1b	2.58961839	0.002762568
Sspn	2.58675237	0.015013314
Gdap111	2.58446104	0.042010661
Tmem255a	2.57903553	4.55639E-07
Sh3rf3	2.55311261	0.00123892
Pipox	2.55237606	0.0035214
Prokr2	2.54834849	0.000884339
B3galt1	2.50824272	0.00062887
Clec14a	2.495834	1.60049E-20
Slc12a8	2.48508379	0.028101691
Zmynd15	2.4581337	0.000147643
H2-Q4	2.41962659	0.012238072
Tmem154	2.41479517	0.006344249
Eng	2.40375217	1.18852E-20
Ahrr	2.40185551	0.024671776
Ccdc110	2.40159921	0.047583515
Il13ra1	2.39997595	0.000174021
Itga2	2.38701229	0.001931815
9630013A20Rik	2.37734364	0.033826138
Fam78b	2.36416915	0.001063811
Cadps	2.36025135	0.046676127
Mrgpre	2.35622134	0.003300233
Gimap6	2.35545309	0.000497308
Pi16	2.35324086	2.97701E-05
Hgf	2.34764215	0.039717815
Plekhg1	2.34189511	4.95345E-11
Cntn1	2.32195392	0.018974153
Cxcl16	2.31527239	2.17805E-06
Cnr2	2.30573281	0.003227649
Edn1	2.29334971	1.65091E-37
Emcn	2.2678929	1.20303E-09
Cldn5	2.26712542	0.01179793
Apba1	2.25723727	0.00481876

Rnft2	2.25079995	0.00099635
Mdfi	2.24735098	0.002126928
Madcam1	2.24125549	7.8355E-07
Patl2	2.23629909	0.00515282
Nod1	2.22755616	0.017552353
D030045P18Rik	2.22492453	0.024330801
Sqrdl	2.22463208	0.01064127
Rgs16	2.2179511	1.7526E-06
Ddx60	2.21568307	0.038891191
Prickle2	2.21311122	5.94511E-14
Ptpn3	2.1918109	0.005325232
Sgk3	2.17972228	1.70099E-10
Cybrd1	2.17942797	0.036779379
Nfil3	2.17816349	9.43137E-05
Adam11	2.17718593	0.005319159
Gcnt4	2.16602276	0.00761664
Serpina3h	2.16119221	0.039303536
Dmrt3	2.15965709	0.022820026
Sparcl1	2.15468423	0.000784538
Adhfe1	2.1482248	0.007585006
Six3os1	2.14355335	4.33993E-05
Nrxn3	2.14053022	0.00347995
Slc35f1	2.11849147	4.76936E-05
Arhgap26	2.11760508	0.016344227
Pcdh17	2.10986521	0.002889373
Sh3gl3	2.10505529	0.034707678
Astn1	2.10033507	0.021058067
Cav1	2.09925614	2.47467E-12
Tnfaip8l3	2.09249146	0.007404196
Tchh	2.08068758	0.02013666
Tm4sf1	2.07157777	0.021439509
Oasl2	2.0665172	0.0320633
Chrdl1	2.06647352	0.015290546
Tnfrsf23	2.05117221	0.003416577
Pcdhga6	2.04796979	0.017445656
Gpr116	2.04274007	2.62151E-12
Fam181b	2.03893273	0.004535041
AI661453	2.02767285	0.011431788
Col14a1	2.00867795	0.014440187
Stx1b	2.00249944	0.023957118
Parp14	2.00240406	0.000385376
Cdh13	1.98465913	0.031087599
Map3k8	1.9826497	0.024648589
Pcdhb17	1.97473016	0.048005393

Qpct	1.97061125	0.010734992
Sost	1.95634788	1.97168E-06
P2rx7	1.95587017	0.005716443
Stc1	1.95258907	0.000216839
Cyrr1	1.94995899	7.75173E-07
Ace	1.94926618	0.000154083
Zbtb7c	1.94059935	0.024431012
Serpina3i	1.93230914	0.026420093
Mmp15	1.93131858	2.63802E-05
Rassf4	1.9211141	0.041461583
Nr2f2	1.91962709	5.46909E-05
Foxp2	1.905238	0.001840115
Rtn1	1.89810313	0.001200266
Stab1	1.89248725	2.62151E-12
Pdzd2	1.88789589	1.49745E-13
Nos3	1.88237258	0.000100201
Bend7	1.8759542	0.001789848
Gpr182	1.87323757	0.013116394
Dmd	1.87144785	3.33051E-06
Ctnnb2	1.86703161	3.99574E-07
Lor	1.86532742	0.01963896
Rab3b	1.85978302	0.00047339
Rnd1	1.85755109	0.041040905
Plekha6	1.85140994	0.031042368
Pde10a	1.84042583	0.010050368
Psmb8	1.83743045	0.027786063
Lhfpl2	1.83134969	1.4092E-06
Kcna6	1.82511296	0.000713988
Gap43	1.82032402	5.1548E-21
4933412E12Rik	1.81999082	0.035906793
Reep2	1.81576473	0.000671371
Nrxn2	1.81220385	0.019511153
Efna1	1.80689469	0.000608615
Ptptr	1.79154996	3.67473E-06
Rgs7bp	1.78985215	0.001521679
Cd93	1.78781028	1.47462E-09
Sh3bp5	1.78110259	5.89188E-08
Slc1a2	1.77465572	2.90794E-07
Gata4	1.77361085	1.4264E-11
Zfp658	1.76190117	0.006038733
Frem1	1.76071677	0.005881318
Dach1	1.75932814	0.043196035
Prex2	1.75415238	2.71593E-11
Xaf1	1.7535399	0.014937183

Tmem86a	1.75231894	0.030928756
Aqp1	1.74957919	1.0118E-05
Whrn	1.74534522	0.037114597
6330419J24Rik	1.74351345	0.004789365
Lgr6	1.73169891	0.000136049
Jag2	1.72948941	0.001174005
Mmrn2	1.72862416	6.0269E-06
Gpm6a	1.72616942	5.13097E-09
Dock4	1.72485324	1.76263E-08
Tmem44	1.7247415	0.002530932
Tmem204	1.72410478	7.78332E-12
Filip1	1.71678234	9.92226E-05
Fes	1.7156733	0.001740874
Gpm6b	1.71382644	6.7478E-08
Tshz3	1.70197523	0.001896077
Mansc1	1.69808565	0.002520927
Pcdhga3	1.69647693	0.006793952
Eml1	1.68342858	2.31344E-11
Cd97	1.68075811	2.15219E-13
Skida1	1.67905073	0.049871418
Grin3a	1.67260577	1.27927E-05
Bace2	1.67258507	7.14205E-05
Rtn4rl1	1.67004782	0.035545165
Ptpm	1.67001425	5.32352E-16
Hpgd	1.66644196	0.046158644
Ocln	1.66423534	0.003780024
Ago4	1.66418307	0.033067184
Sox17	1.66352056	4.86361E-17
Bmf	1.65824119	7.16797E-07
Plcd1	1.65246216	0.005508605
Arhgef28	1.64188196	0.004154036
Ccdc120	1.63713556	0.003731318
Gnai1	1.63104051	2.78741E-12
Epas1	1.62674831	1.54656E-16
Nrep	1.6241192	1.60062E-20
Dhrs3	1.61872683	4.9319E-05
Fzd1	1.61781646	3.26112E-08
Adcy4	1.61753975	5.52292E-10
Slc40a1	1.60771557	7.62751E-05
Kctd12b	1.60298537	7.39103E-08
Mturn	1.59050795	0.016730548
Ndrp2	1.58502795	0.012945763
Klhl8	1.58422961	0.001909046
Tnnt2	1.57898015	0.005477122

Met	1.57897949	0.000141575
Gm20748	1.56772455	0.000484413
Naalad2	1.56349374	0.011969237
Eltd1	1.56326302	3.64109E-07
Cxx1a	1.56195873	0.009435987
Dgkk	1.55921393	1.7379E-05
1810011O10Rik	1.55803329	2.83423E-06
Npdc1	1.55318398	0.007309947
Slc16a2	1.54823787	1.38332E-13
Lrrn2	1.54572792	0.000118107
Id2	1.53972139	5.81204E-09
Pdk4	1.53886892	0.009747462
Sema3a	1.53813239	1.66242E-06
Lrrk2	1.53395858	1.67821E-10
Ppp1r16b	1.53365855	5.76626E-09
Trim47	1.52365175	0.007798689
6430548M08Rik	1.517959	0.003489248
2510009E07Rik	1.50602202	0.000267407
Dok4	1.50596452	0.000513324
Oit3	1.5047518	0.000127365
Itpr3	1.50208177	2.87372E-06
Ankrd6	1.50197519	0.023957118
St8sia4	1.49910183	0.020200454
Cyp39a1	1.49807854	0.044400618
Erbp2	1.49752692	6.00543E-11
LOC102636514	1.48547575	0.003300233
B630019K06Rik	1.48122346	0.041391858
Adcy2	1.48104462	0.029844293
Tm6sf1	1.47720907	1.47462E-09
Fxyd5	1.47565629	0.008119747
Rgs2	1.47522281	1.38332E-13
Ramp2	1.4730347	6.55888E-06
Ube2l6	1.46985536	0.01150381
Emb	1.46430329	6.98299E-07
Angptl2	1.45622852	0.00019519
Klhl4	1.45505394	0.000394479
Cish	1.45430575	0.014270424
Rab15	1.4529967	0.022289537
Hecw2	1.45168155	3.97907E-08
Gimap8	1.44923421	0.003171631
Akr1b8	1.4480994	0.000354722
Rgs3	1.44669161	2.79034E-07
N4bp3	1.44325245	0.001014064
Nedd9	1.44126253	0.000682966

Acer2	1.43991526	0.001950725
Kctd21	1.43725253	0.017552353
Parp8	1.43446184	1.16695E-07
Pcdh12	1.43441496	8.06968E-14
Chst11	1.43216905	0.000491398
F8	1.43126819	8.64663E-06
Cpt1a	1.43019018	9.66042E-05
Hoxa1	1.42944378	0.036779379
Ctsl	1.42932441	2.78741E-12
Limch1	1.42881176	3.30849E-06
Gimap4	1.42612128	4.80559E-07
Acvr11	1.41594839	3.91822E-08
Ptchd1	1.41513888	4.9372E-07
Gabrb3	1.41110383	0.001412391
5730409E04Rik	1.41066928	0.025586714
Bmp6	1.40916486	1.00348E-09
Tgfbr2	1.39565726	2.07504E-06
Cubn	1.3950718	0.012238072
Notch4	1.39434678	4.55768E-05
Ube2q11	1.39284044	0.047761325
Upp1	1.39204852	3.57082E-06
Lpar4	1.38897623	2.34423E-09
Dab2	1.38709916	0.001707355
Icam1	1.38513664	5.82923E-05
Icam2	1.38476952	0.000439071
Rasgrp3	1.38203471	2.62151E-12
Mcam	1.38184493	6.96898E-06
Ehd2	1.38165765	1.28373E-07
Gprc5a	1.3813017	0.007857311
Prkch	1.38094944	0.004465313
Scrn1	1.37909994	0.025410734
Ctsh	1.37331548	0.000942925
Nav3	1.37169696	0.02505003
At11	1.37126786	1.29486E-06
Slc8b1	1.37035742	0.005082306
Spred3	1.36909934	0.01482001
Antxr2	1.36625734	0.004418737
Grp1	1.3648216	0.000603886
Sema6d	1.36347571	5.29662E-08
Rgmb	1.36015967	0.004766197
Parvb	1.3598599	0.000213304
Sema3f	1.35666009	0.002533692
Lonrf3	1.3528561	7.2611E-06
Tlr4	1.34398922	2.0982E-05

Capn5	1.34397931	0.000152011
Cttnbp2nl	1.34265528	1.08006E-19
Adam12	1.34233974	0.000623682
Itpkb	1.33568824	2.03771E-05
Bgn	1.33373234	1.31763E-07
Pld1	1.33141477	7.46474E-07
Cyp4v3	1.32340215	0.010515006
Plagl1	1.32121121	0.000761453
Arid5b	1.31915449	2.34423E-09
Sgsh	1.31853498	3.23703E-05
Rcan3	1.31116263	0.000190286
Kctd17	1.31029115	5.46315E-06
Pdk1	1.30864086	0.000946184
Helz2	1.30753826	0.011276152
Atf3	1.30473364	4.0506E-08
Itga1	1.30115946	9.11806E-09
Fmo1	1.29472153	1.14916E-16
Adam23	1.29284152	1.69365E-06
Sox6	1.29150052	1.00407E-07
Prom1	1.2904929	0.015321403
Ppm1j	1.28898246	0.047736329
Slit2	1.28258055	0.00032164
Plvap	1.27866926	0.000509457
Fmo5	1.27415737	0.021229611
Tmem159	1.2686847	2.48102E-05
Gbp9	1.26835918	0.027665722
Psen2	1.26813081	0.019284155
Tmem173	1.26774222	0.013769531
Plcb1	1.26498936	3.06527E-05
Chrnbl	1.26357764	0.024844203
Ehd3	1.25677014	0.01482001
Cd40	1.25564798	1.04773E-05
Kdm7a	1.25316491	6.67987E-08
Hmcn1	1.24948206	4.9372E-07
Pcdh1	1.24821296	1.22328E-06
Timp1	1.24713649	0.043937948
Ppap2a	1.24317985	0.002303112
Dock8	1.23892721	0.000148873
Tfec	1.23820736	4.36716E-07
Dusp22	1.23817215	3.64109E-07
Rps6ka2	1.23780729	2.57761E-06
Mical2	1.23514769	0.000331948
Osbp16	1.23223577	0.03023915
Rab3il1	1.23010403	0.008988872

Efnb2	1.22584467	2.74734E-12
Sat1	1.22396658	1.49832E-10
Sgk1	1.21928875	2.69762E-08
Fgd6	1.21646329	6.18775E-05
Rarg	1.21113647	0.012145624
Entpd1	1.20279758	0.035626622
Dnajb5	1.20061997	0.004435643
Ntn3	1.19496915	4.14625E-05
Rnf144a	1.19357888	4.43976E-05
Arsa	1.19298159	0.002530932
Arhgef15	1.18893214	7.14205E-05
Eps8	1.18844868	0.000134139
Tnks1bp1	1.18720037	0.001125329
Cerk	1.18674938	0.005050143
Gbp4	1.18608431	0.01858609
Ly96	1.18476099	0.045726424
Dennd5b	1.184206	7.51897E-07
Fbx17	1.18064294	0.000529909
Elovl4	1.17998447	0.000442539
Tgfb1	1.17819143	0.030828711
D1Ert622e	1.1708281	0.01179793
Gatm	1.16890665	0.002303112
Rgl1	1.16709318	1.04659E-11
Tie1	1.16496502	0.012145624
Ctgf	1.16010458	0.002370819
Cbx6	1.15982767	0.025410734
Epn2	1.15805733	3.92099E-12
Rnf165	1.15781922	0.00341342
Prdm1	1.15756418	0.019634188
Dysf	1.15635916	0.001690251
Usp27x	1.15446047	0.020712294
Itsn1	1.15339098	2.74734E-12
Cdh5	1.14956573	0.007024169
Mecom	1.1474593	0.00046904
Dtx3l	1.14619799	0.004151397
Ghr	1.14443005	1.16037E-10
Mfap3l	1.14063473	0.001320575
Rhpn2	1.13471415	8.84809E-05
Slc9a3r2	1.13254832	8.27541E-06
Filip1l	1.1321366	2.35311E-05
Tnfaip1	1.12721741	4.35839E-07
Sema6b	1.12467937	0.043443023
Rhou	1.1222825	8.03697E-08
Snrk	1.12179423	0.000265586

Plekha1	1.11979284	5.76626E-09
Stap2	1.11946019	0.000776078
Arhgef3	1.11938832	0.001529963
Fat4	1.11909352	3.34289E-07
AW551984	1.11908907	0.007970266
Ccdc80	1.11603822	2.42344E-05
Tmc8	1.11280255	0.031340642
Ccnjl	1.11244286	9.29763E-06
Ypel2	1.11039245	0.001306038
Abhd6	1.10911504	0.006734222
Sash1	1.10646508	1.34103E-08
Ampd3	1.10612842	0.000568351
Endod1	1.10538157	0.001393788
Sox18	1.10425759	0.025088085
Pced1b	1.1009767	0.004942393
Parp9	1.10007273	0.022590568
Parp10	1.09553229	0.046324916
C1qtnf6	1.0943025	0.003161654
	40422	1.0923483
Zfp382	1.0912224	0.0406944
S1pr1	1.08836574	5.18886E-09
Acsf2	1.08560817	0.003227649
Mdfic	1.08416514	0.001233198
Trim16	1.07769101	0.001032334
Ptprd	1.07514088	8.71795E-07
Heg1	1.07463882	0.00010943
Glul	1.07355443	7.25498E-11
Flrt2	1.07101031	1.05183E-09
Armex1	1.06964681	1.16037E-10
Nipal3	1.06883569	0.044565478
Sft2d2	1.06801998	3.96543E-07
Gpr126	1.06463852	0.047141713
Fbn1	1.06250049	4.67821E-06
Fstl1	1.05755394	4.3809E-09
Zfp618	1.05376379	0.028880357
Cers6	1.05354182	0.010272676
Prickle3	1.05255551	0.012461136
Klf4	1.05032488	3.34249E-07
Arhgap31	1.04630502	3.25179E-05
Ap1s2	1.0448927	9.42993E-07
Gja4	1.04113201	0.043941057
Hand2	1.03844154	2.03771E-05
Asb4	1.02904765	0.026253214
Mxi1	1.02840984	0.000569955

Slc17a5	1.02556749	0.009523916
Bcl2	1.02445677	0.00882067
Tlr3	1.02388561	7.66505E-05
Rhob	1.02378506	0.000358687
Meis2	1.02254553	0.019922414
Gpr146	1.02105459	0.011179574
Fam114a1	1.02104151	6.58615E-08
Cd109	1.02066387	0.015844706
Vash1	1.01960591	0.000539094
Lmf1	1.01835716	0.047145219
Peli2	1.017516	0.001315988
Maf	1.01633322	0.044860977
Mroh1	1.01533484	0.00173172
Plat	1.01210985	0.025681478
Trim2	1.00916577	0.028999329
Tcf4	1.00896003	1.76263E-08
Rcsd1	1.00162662	0.014440187
Thbd	1.00146299	0.03023915
Slc26a10	1.00080202	0.002174643
Elk3	1.00021479	1.28227E-05
Cpne8	0.99605113	0.024692245
Rusc1	0.99581914	0.039975742
Peg10	0.99468146	1.01047E-05
Lipa	0.99340069	1.24628E-07
Pdgfb	0.99193709	0.001178785
Tmem2	0.99150421	3.05746E-06
Prrg3	0.99149258	0.000596048
Atg10	0.99136987	0.019634188
Kank1	0.98701352	0.007340664
Myct1	0.98380908	0.023446821
Fblim1	0.98165366	0.000130731
Pitpnc1	0.98138227	0.001690251
Camk2d	0.98066506	2.17158E-05
Spats2l	0.97784568	0.000264676
Plp2	0.97773516	1.0606E-09
Phyh	0.97773444	0.027919545
Pik3r3	0.97521746	3.45092E-06
Anxa5	0.97421157	4.41668E-10
St3gal6	0.97298244	0.001053936
Bcl6b	0.97270841	0.00912701
Arap2	0.97066286	0.000237722
Plod1	0.97031481	1.95941E-07
H2-D1	0.97008472	0.018813997
Ushbp1	0.96956833	0.003222281

Cd55	0.9675418	0.000195384
Id1	0.96676281	1.91118E-06
2900026A02Rik	0.96611254	6.67852E-06
Kif3c	0.962524	0.002407405
Smad6	0.96122668	0.001840092
Gnal	0.95992629	0.01006963
Marveld1	0.95928409	0.000491459
Asap2	0.95866243	1.61566E-07
Dusp1	0.95834233	1.95941E-07
Slc5a3	0.95613095	8.27149E-06
Vim	0.95304285	1.14278E-08
Slc16a13	0.95140597	0.021745481
Slc43a3	0.95098653	0.00252015
Bmper	0.94912401	0.025921359
App	0.94760363	0.000198842
Otud1	0.94066555	0.019591334
Wbscr27	0.93455272	0.025668121
Leprel2	0.93089559	0.025464706
Ece1	0.92701377	8.23016E-07
Acvr1	0.92537402	0.001690251
Zeb1	0.92283183	0.000413386
Prnp	0.92205661	0.006862971
Atp11a	0.91993892	9.81911E-08
Crybg3	0.91509299	0.000399004
Pqlc1	0.91466376	0.01323112
Plxnd1	0.91335683	3.94278E-06
Rasip1	0.91306805	0.031221634
Slc6a6	0.91284416	1.05547E-06
Asah2	0.91209642	0.000475057
Ndrgr1	0.91198646	0.042754269
Mboat2	0.9107657	0.003756264
Hexb	0.91047392	0.018134441
Apbb2	0.90950282	0.000638892
Dcbld1	0.90927245	0.015219914
Myo6	0.90924173	0.002158363
Nfkbia	0.90666537	0.029404986
Edil3	0.90292285	0.02174393
Mcc	0.89900153	4.53883E-05
Znfx1	0.89539114	0.004942393
Armxc4	0.89529063	3.74857E-11
Ankrd44	0.89363822	0.011299418
Plk2	0.89271298	2.3896E-06
Jup	0.88980737	1.0674E-06
Prkce	0.88971728	0.000242988

Trib1	0.88855817	0.000164087
Sirpa	0.88847172	0.0032421
Sh3rf1	0.88714516	7.95249E-05
Herc3	0.88713647	0.02040009
9430020K01Rik	0.88711749	1.00867E-05
Nfib	0.88684759	0.006734222
Bcl2l11	0.88607971	3.79149E-07
Nagk	0.88524847	0.001014064
Peg3	0.88415645	0.001693132
Ttc28	0.88037653	0.00047339
Tbc1d9	0.87763921	0.005050143
Lcp1	0.87577972	0.017552353
Fbxo10	0.87553724	0.000484413
Dhx57	0.87543092	9.81911E-08
Tor4a	0.87431145	0.014140212
Tanc1	0.87249721	1.15752E-07
Fgd5	0.87024341	5.29662E-08
Rras	0.87010233	3.52916E-05
Klhl13	0.86815254	1.01595E-05
Klf2	0.8669513	0.000232828
Fam69a	0.86321708	0.010592548
Wsb2	0.8629341	0.000353186
Rims2	0.86275048	0.015219914
Dusp6	0.86100696	7.86127E-05
4931406P16Rik	0.85821535	1.14463E-06
Gng11	0.85577535	0.00010029
Col4a1	0.85572026	1.27974E-08
Fyn	0.85458237	0.030073173
Vegfc	0.85317872	0.00912701
Mllt6	0.85243076	5.50239E-05
Tmem176b	0.85234919	0.00759766
Dgkh	0.85065338	0.005557702
Id3	0.84983369	8.69853E-07
Ltbp1	0.84714648	0.01858609
Klf6	0.84618231	0.007412582
Procr	0.84381173	0.021201829
Jag1	0.84337645	0.04692825
Zfp532	0.84197757	4.20211E-05
Tapbp	0.83940005	0.016952314
Col4a2	0.8384229	1.59634E-07
Dlc1	0.83789397	1.50457E-07
Creb3	0.83774535	0.008378562
Nid1	0.83665967	0.016884793
Pcdhgc3	0.83645238	0.003073471

Tspan18	0.83628395	0.032760213
Arhgef5	0.8333038	0.001135402
Tmc6	0.83177248	0.014936968
Cdr2l	0.83123085	0.000689689
Gpr137b	0.8308372	0.01399787
Amotl1	0.83002043	2.79154E-05
Arhgap27	0.82950263	0.001339082
Lpar6	0.82549977	0.002249377
Osbpl3	0.82535908	0.039860331
Ermp1	0.82500645	5.20026E-05
Unc13b	0.82464396	0.004253621
Cd38	0.8242372	0.005069433
Rin2	0.8241474	0.01146119
Lrrc8c	0.82394381	0.000134139
Palld	0.82388517	0.000702081
Ralgapa2	0.82382984	0.013376559
Rragd	0.82216875	0.009409538
Hyal2	0.82155336	0.001091906
Creb3l2	0.82147014	7.94869E-05
Zfp462	0.82053886	0.035502939
Btbd3	0.82036163	0.00057821
Nxpe3	0.81835438	0.007625924
Hspa12a	0.81816773	0.000497308
Spag9	0.81544543	6.30049E-05
Sh3tc1	0.81403036	0.001037751
Pam	0.81334126	3.52916E-05
Pim3	0.80868711	0.003911677
Pros1	0.80804587	0.000529909
Fam115a	0.80737277	0.000643508
Anxa3	0.80582012	0.00759766
Notch1	0.80494113	3.83317E-05
Crmp1	0.80486172	0.02670682
Rasal2	0.80465513	3.53388E-05
Sparc	0.80441467	0.000108353
Mef2a	0.80342491	0.003423088
Nhs1l	0.8027688	0.00046271
Camk4	0.8025306	0.005352197
Tek	0.80198983	0.025731459
5031439G07Rik	0.80153495	0.022986871
Tbc1d12	0.80113584	0.024554354
Dcxr	0.79980701	0.014862217
Kdr	0.79919545	0.000147643
Pde1c	0.79849955	0.035981062
Klf9	0.79835413	0.049000364

3110043O21Rik	0.79722091	0.045899837
Hsd17b11	0.7968532	0.008376881
Wwtr1	0.79563496	3.1261E-06
Mtch1	0.79150964	4.57888E-07
4930402H24Rik	0.78917036	0.003071407
Ankrd50	0.78902273	3.65637E-06
Ppp3ca	0.78851049	7.03897E-05
Pgap1	0.78722922	0.011690857
Man1a	0.78141342	0.000204243
Ezh1	0.7813293	0.000650618
Serpinf1	0.77974119	0.020078977
Fam43a	0.77910444	0.000544414
Lcorl	0.77650167	0.000106664
Cntln	0.77580148	0.034255696
Pvr	0.77573143	0.0007004
Pcyt1a	0.77081897	0.001756583
Rnf157	0.76917696	0.000185175
Srgap1	0.76881999	1.88927E-05
Unc5b	0.76821385	0.023645943
Gpr19	0.76784159	0.022322881
Adarb1	0.7638246	0.019634188
Crip2	0.76264077	0.000603523
Enox2	0.76209522	0.003227649
Tnc	0.76201871	0.012079092
Myo1b	0.75987452	0.001370017
Lama4	0.75747045	0.013495977
Palm	0.75488996	0.010068361
Mef2c	0.75433857	0.047869168
Obfc1	0.75209303	0.000770405
Gsto1	0.75191683	0.048700709
Adam15	0.75179566	0.000459628
Clip2	0.75089598	0.012250073
Rilpl1	0.75064451	0.023134393
B2m	0.75057654	0.000235722
Crip1	0.75031877	0.002889373
Mageh1	0.74814737	0.014737269
Prr5l	0.74490322	0.034017929
Atp2b4	0.7420747	0.046466866
Ets1	0.7412154	0.000303905
Hpcal1	0.73952115	0.012987777
Pecam1	0.73847653	0.037844023
Pbxip1	0.73775555	0.005000647
Camsap2	0.7373141	0.009287807
Elmo1	0.7370861	0.005507327

Pcnx	0.73615789	3.94535E-05
Tmem176a	0.73606916	0.036898442
Srr	0.73201569	0.02533065
Akap12	0.73179423	0.013115436
Vegfa	0.73158639	0.004535041
Uap111	0.73105184	0.002278496
Hykk	0.72645343	0.000945975
Ephb4	0.72564068	9.43137E-05
Man2a1	0.72442335	2.48102E-05
Rab11fip3	0.72286423	0.000264676
Dse	0.72239888	0.017934181
Klf3	0.7220224	0.000852713
Gria3	0.72143183	0.011027841
Renbp	0.7185534	0.002433371
Vwa5a	0.7183543	0.000185665
Arhgef12	0.71826823	2.64054E-07
Dscr3	0.71820235	0.013373111
Klf12	0.71801859	0.042038097
Mest	0.71748714	0.02435718
Add3	0.71507281	0.012250073
Tnip1	0.71391741	0.046323861
Foxp1	0.71179306	0.007440962
Agtppb1	0.7110216	0.047479631
Atxn1	0.70765713	0.047746792
Ublcp1	0.70538133	0.001442801
Slc36a1	0.70482982	0.034707678
Ecsr	0.70482623	0.00114924
Nfkbiz	0.70403005	0.03182126
Ltbr	0.70389335	0.013710817
Ift122	0.70383341	0.0163981
Ptprb	0.70281707	0.012058309
Ehd4	0.70160983	0.001007446
Shroom2	0.70152849	0.002080608
Caskin2	0.70133712	0.000825178
Jun	0.69962255	0.001690251
Colec12	0.69953303	0.00064055
Usp29	0.69942113	0.021813981
Dcaf12l1	0.69936957	0.030760077
Thsd7a	0.69934142	0.006517304
Ralb	0.6988076	6.72988E-05
Rffl	0.69851775	0.028467784
Gcc1	0.69814953	0.036533027
Diap2	0.6974635	0.000107239
Hivep1	0.69726877	0.001151491

0610031J06Rik	0.69448255	0.001056958
Nrp1	0.69364745	0.000413386
Hdgfrp3	0.69162281	0.017150534
Pdlim1	0.69026258	0.00588207
Cmtm6	0.6882245	0.007776813
Tbc1d24	0.68781396	0.000761453
Grina	0.68632164	0.03875513
Ssfa2	0.68422394	0.000529909
Slc31a2	0.68153524	0.030759745
Crebrf	0.67763436	0.01150381
Rab31	0.67591862	5.17294E-05
Nfatc1	0.67566145	0.000358201
Ankrd46	0.6737142	0.014225415
Eogt	0.67287239	0.000279617
Tmem136	0.67127123	0.020783337
Tmtc2	0.67104799	0.02269917
Zfp423	0.66883462	0.000868266
Nhsl2	0.66800437	0.045635912
Phactr2	0.66526633	0.000168442
Aplp2	0.66374113	0.002158363
Plxnc1	0.66342244	0.046158644
Zfp37	0.66136321	0.046466866
Maged2	0.66117415	0.001690251
Gdpd1	0.6590052	0.012339667
Spats2	0.65873136	0.012461136
Abhd17a	0.65611991	0.042241708
Rnf19a	0.65543242	0.011892489
2810025M15Rik	0.65526736	0.03018424
Trerf1	0.6552643	0.032909333
Phldb1	0.65390566	0.033454148
Cmtm3	0.65378612	0.002099005
Map4k5	0.65273535	0.000682966
Rftn1	0.65088843	0.007188629
Mapre2	0.65072114	3.04588E-05
Car13	0.6501821	0.029781297
Rap2a	0.6495413	0.00012727
Sorbs1	0.64864152	0.005769709
Anxa6	0.64795673	0.005050143
Aldh3a2	0.64686485	0.007871539
Trp53inp2	0.64632375	0.006543975
Dock11	0.64291223	0.001077678
Fermt2	0.64276518	0.001317511
2010111I01Rik	0.64226543	0.023531808
Kdelc2	0.64148983	0.032128846

Rnf185	0.64126914	0.006954703
Cpped1	0.64121622	0.020014143
Acox1	0.64033263	0.003251755
P4ha1	0.64031227	0.023531808
Ucp2	0.63959579	0.013484556
Litaf	0.6387186	0.000584105
Klhl24	0.63412857	0.000617457
Ddx26b	0.63165374	0.000923543
Tbx3	0.6314738	0.01128909
Neo1	0.63074852	0.001151491
Dcaf6	0.62954061	0.02505003
Tmbim1	0.62939469	0.003961101
Sin3b	0.62730055	0.00687311
Ctns	0.62718535	0.024164708
1700017B05Rik	0.62710297	0.006939776
Stk39	0.62697845	0.029616385
Pabpc4l	0.62383771	0.039717815
Psap	0.62363956	0.002904454
Bcor1l	0.62358615	0.044217964
Rcan1	0.62350671	0.007890401
D8Ert82e	0.62298176	0.020080206
Pald1	0.62157232	0.004155895
Hspg2	0.62080375	0.023583472
Mfsd1	0.62021811	0.014816049
Tram2	0.62013336	0.017156973
Timp3	0.62007929	0.045270638
Large	0.61708174	0.00070527
Mrps18a	0.61666294	0.01963896
Ap1b1	0.61648275	0.026554312
Atp7a	0.61478233	0.000656631
Cdk19	0.61444285	0.003519578
Ddah2	0.61139152	0.000539094
Pcmt2	0.61134989	0.041391858
Wdr59	0.61015405	0.001261071
Txndc12	0.60745129	0.017302164
Sh2d3c	0.6070239	0.046485043
Ctsb	0.60682587	0.002415398
Coro2b	0.60484299	0.015178259
Sntb2	0.60408587	0.023485284
Dram2	0.60395019	0.003681232
Snx33	0.60357863	0.042833276
Ctsz	0.60319387	0.042038097
Dpysl3	0.60275999	0.023848921
Hbp1	0.60202876	0.001246888

Pygb	0.60159009	0.024938826
B4galt1	0.60021312	0.009472726
Chst2	0.59935197	0.044317282
Itm2a	0.59934598	0.016952314
Fam3c	0.59884264	0.002171325
Lrp5	0.59853274	0.004574116
Dst	0.59845677	0.04029844
Tjp1	0.59775217	0.002024504
Tulp4	0.59769468	0.042038097
Ankrd12	0.59745828	0.031663064
Magi3	0.59609837	0.000744992
Cds2	0.59605618	0.003453483
Agap1	0.5959778	0.000824873
Snx30	0.59496096	0.001178785
Tirap	0.59184831	0.04215328
Laptm4b	0.59147045	0.016153005
Nckap1	0.59012864	0.000861761
Trio	0.58716907	0.001049105
Ccser2	0.58667636	0.012536686
Ctsa	0.58558901	0.002521886
Zfp365	0.58450017	0.010050368
Mrps6	0.58421779	0.015953899
Dip2a	0.58319374	0.012216594
Msn	0.58263225	0.002146891
Fuca1	0.58182629	0.030945311
Myl12b	0.58058091	0.003073471
C1galt1	0.58014878	0.044217964
Arhgap32	0.58007553	0.019465196
Cd81	0.57997559	0.002473598
Bin1	0.57985951	0.022188608
Ppm1f	0.57934618	0.009875557
Slc35f5	0.57639734	0.011713162
Frdm4b	0.57613481	0.001740874
Nbea	0.57567119	0.013359638
Fam149a	0.57540988	0.031478578
Gas6	0.57495921	0.024093476
Ttc21b	0.57463453	0.047145219
Cflar	0.57426542	0.010484254
Tet2	0.57397044	0.021805972
Abca3	0.57208283	0.039347379
Cdc42bpb	0.57079366	0.002730013
Kank2	0.56893831	0.000687737
Pea15a	0.56454173	0.007986298
Tmeff1	0.56339126	0.002796434

Map4k3	0.56184021	0.007739628
Calcoco1	0.55926819	0.021813981
Lphn2	0.55915459	0.028792274
Frmd4a	0.55909661	0.009381743
She	0.55727128	0.014278091
Bex2	0.55682153	0.017018905
Fam222b	0.55582617	0.003171631
Tjp2	0.55518464	0.047736269
Gabarapl1	0.55411434	0.027968234
Bcl2l2	0.55379105	0.025731459
Macf1	0.55359979	0.008348814
Cyth3	0.55158306	0.017107797
Tcf7l2	0.54950471	0.019670119
Mapk3	0.54926569	0.012042675
Sypl	0.54779776	0.002164505
Casd1	0.54730099	0.004621678
Ndel1	0.54515163	0.032707262
Elovl5	0.54455376	0.007198543
Zfyve1	0.5429496	0.034501245
Gm16515	0.54253368	0.030750353
Oxct1	0.53889576	0.003227649
Hexa	0.53755868	0.010353402
Hip1	0.53669525	0.003779115
Akt3	0.5358331	0.002640916
Ctnnb1	0.53480571	0.002134974
Gna12	0.53476745	0.036457407
Tmem106b	0.53293082	0.00983404
Kctd10	0.53232996	0.005050143
Dlg5	0.53052194	0.026201187
Copg2	0.52907354	0.007441806
Klhl6	0.52885112	0.043764027
Bmpr2	0.52816564	0.012806133
Arhgef11	0.52804764	0.04460364
Tenc1	0.5270311	0.046485043
Exoc4	0.52598929	0.013850109
Grn	0.52512264	0.017073221
Sccpdh	0.52503297	0.020276582
Asah1	0.5240867	0.004107884
Adipor1	0.52219677	0.027909075
Cd34	0.52113702	0.044968769
Sptbn1	0.52107557	0.011841617
Dmwd	0.51969525	0.034731938
Steap2	0.51959855	0.016884793
Acbd5	0.5192529	0.010635018

Fam129b	0.51726306	0.045007815
Sri	0.51476517	0.027981567
Myo1c	0.51023212	0.026664828
Anapc2	0.5094904	0.047761325
Clip1	0.50854321	0.045970845
Usp32	0.50601689	0.006862971
Lrrc58	0.50208572	0.014354849
Lrrc8a	0.50131466	0.015484623
Numb	0.50039981	0.038467385
Tgoln1	0.49960455	0.020080206
Hdac7	0.4984747	0.021512228
Yipf3	0.49814051	0.04568778
Rab12	0.4976599	0.016680184
Lamp2	0.49504798	0.00331656
Cdh2	0.49261398	0.044437726
Slk	0.49239968	0.04613748
Cobll1	0.49228499	0.046238274
Sptan1	0.4915641	0.006608602
Ctsd	0.49134742	0.006278841
Slc44a2	0.49073396	0.014312804
Nav1	0.48608593	0.012488255
Pxdn	0.48546871	0.047497123
Dock6	0.48544075	0.026055774
Cnppd1	0.48498683	0.036442504
Srgap2	0.4838278	0.015024717
Mpzl1	0.48375674	0.004183166
Smad1	0.4829588	0.0470535
Il6st	0.47740513	0.016300121
Pik3c2a	0.47232209	0.020342359
Ppp1r9a	0.47205388	0.032031301
Fkbp9	0.47062321	0.031063557
Lgals8	0.47012918	0.037800364
Atp2b1	0.46992678	0.005771646
Npc1	0.46778791	0.030749983
Atp2c1	0.46589562	0.02782276
Yes1	0.46384103	0.032301302
Tspan9	0.4601918	0.019634188
Nrp2	0.45803467	0.031478578
Dusp16	0.45754214	0.046634435
Iqgap1	0.45699364	0.039120924
Map3k3	0.45523166	0.016592229
Qk	0.45351265	0.031272574
Dock1	0.4495832	0.047761325
Btg1	0.44955823	0.038726639

Actn4	0.44890813	0.010940718
Carhsp1	0.44872969	0.022590568
Adcy6	0.44322494	0.027909075
Ptk7	0.44099911	0.046270724
Itga5	0.43983133	0.019283968
Man2b1	0.43442997	0.020610191
Ugp2	0.4328997	0.035589807
Map3k11	0.43032825	0.034398076
Fam135a	0.42739733	0.04226346
Pdlim5	0.42663236	0.036732568
Triobp	0.41876773	0.02533065
Gng12	0.41696342	0.042038097
Ptpn14	0.41348236	0.039033139
Piezo1	0.40509281	0.036447588
Skil	0.39370361	0.04302612
Map4k4	0.39335155	0.043693615
Lamp1	0.37683457	0.044951032
Maged1	0.37170291	0.041391858
Trim33	-0.3573469	0.046505472
Gspt1	-0.3605518	0.049871418
Sqle	-0.3611902	0.047497123
Tardbp	-0.3666847	0.0450795
Tcerg1	-0.376228	0.04692825
Psmb6	-0.3846357	0.034358015
Hnrnp1	-0.3850724	0.041383915
Orc3	-0.386385	0.049501447
Sf3a1	-0.3926086	0.029792015
Med14	-0.3953839	0.035347688
Ola1	-0.396888	0.030828711
Stip1	-0.3980127	0.025731459
Rrm1	-0.40015	0.048973333
Set	-0.4020976	0.019634188
Pdcd11	-0.4036084	0.036446307
Pkm	-0.4037588	0.033126447
Ncapd2	-0.4068065	0.034632648
Xpo5	-0.4114309	0.043826737
Utp6	-0.4115701	0.031272574
Wdr75	-0.4123941	0.032767466
Tyms	-0.4180273	0.030749983
Mybbp1a	-0.4199431	0.027692569
Smarcc1	-0.4207404	0.010592548
U2af2	-0.4218187	0.036356386
Nup62	-0.4218858	0.03659348
Epb4.115	-0.4222133	0.034933449

Ddx18	-0.4238809	0.019037708
Cops5	-0.4239614	0.047519961
Znrf3	-0.4252158	0.039120924
Cirh1a	-0.4273012	0.025731459
Plagl2	-0.4287221	0.037114187
Tll4	-0.4302274	0.036453576
Rae1	-0.4322049	0.02672746
Fam210a	-0.4326129	0.033370023
Larp7	-0.4335868	0.01353108
Hnrnpc	-0.433706	0.04463755
Cpsf6	-0.4344144	0.031087599
Nsun2	-0.4344325	0.049403296
Nme1	-0.4416846	0.018530298
Aifm1	-0.4417268	0.039476839
Denr	-0.4420595	0.048771943
Mre11a	-0.4431858	0.039975742
Nup160	-0.445214	0.017009718
Nup155	-0.4457544	0.014937183
Psmb3	-0.4494857	0.024434239
Snrnp70	-0.4500929	0.034951497
Smn1	-0.4510802	0.047761545
Fasn	-0.4512933	0.012637806
Plcl2	-0.4526148	0.0450795
Fastkd2	-0.4526201	0.043052075
Dtl	-0.4527959	0.045979705
Gins2	-0.4540335	0.042494308
Ppat	-0.4559212	0.01150381
Fus	-0.4564607	0.0163981
Dhfr	-0.4572799	0.036850504
Atic	-0.457867	0.038996551
Acaca	-0.458071	0.033011588
Mms221	-0.458251	0.043693714
Wdr3	-0.4585454	0.047622621
Utp111	-0.4591145	0.04692825
Hat1	-0.459847	0.039717815
Usp24	-0.4598783	0.013999324
Hells	-0.4618291	0.019912997
Rsl1d1	-0.4630114	0.008975873
Tacc3	-0.4632733	0.023047439
C1qbp	-0.4654797	0.019284155
Khsrp	-0.4659537	0.025432283
Erh	-0.4663217	0.012250073
Nudc	-0.4675083	0.023153375
Eif4e	-0.4700993	0.01482001

Ruvbl2	-0.4714274	0.017155849
Frs2	-0.4737451	0.020031543
Fgfr1op	-0.4764688	0.02174393
Rsl24d1	-0.4794557	0.030886228
Gatad1	-0.4821751	0.004813209
Phb	-0.4823853	0.026336996
Park7	-0.4844912	0.03428394
Tsr1	-0.4870355	0.025432283
Chaf1b	-0.4875325	0.031945563
Myo19	-0.4878522	0.041255062
Trmt6	-0.4884361	0.034951497
Cct3	-0.4887553	0.005374654
Chek1	-0.4912268	0.033067184
Rcc2	-0.4921252	0.014225415
Cacybp	-0.4933062	0.027808796
0610007P14Rik	-0.4934913	0.032181752
Ticrr	-0.4936432	0.015447187
Oat	-0.494166	0.005101356
Ing5	-0.4949474	0.026264805
Psmg1	-0.4960053	0.018685955
Tuba1c	-0.4960215	0.001909046
Cks1b	-0.4983408	0.010068361
Dkc1	-0.500082	0.025502096
Mlec	-0.5010375	0.003793243
Wdr18	-0.5016548	0.027602575
Eef1d	-0.5016561	0.005029593
Gpn1	-0.50166	0.031272574
Kif20a	-0.5021924	0.020251678
Gfpt2	-0.5025914	0.038891191
C330027C09Rik	-0.503346	0.020767104
Rrp1b	-0.5039365	0.004155895
Jade3	-0.5045377	0.04865351
Phlda1	-0.511139	0.015484623
Mrps30	-0.5112446	0.03023915
Nsdhl	-0.5131118	0.004035043
Emg1	-0.514678	0.03023915
Pitrm1	-0.5148078	0.036723138
Dazap1	-0.5151004	0.043443023
Pcna	-0.5155391	0.006109254
Eno1b	-0.5159822	0.027909075
Pspc1	-0.5165724	0.026849481
Cenph	-0.5175012	0.034731938
Srm	-0.5186396	0.012896026
Uhrf1	-0.5191902	0.002298833

Nol10	-0.5216314	0.036225763
Eif5a	-0.5225901	0.000901976
Rfc5	-0.5234656	0.040120088
Seh1l	-0.523609	0.001159918
Mycbp	-0.5236966	0.023238999
Sf3b4	-0.5257505	0.01150381
Gemin5	-0.5259505	0.012360708
Pno1	-0.5261394	0.005017275
Mrpl18	-0.5270604	0.007149373
Hspd1	-0.5276276	0.016206396
Phykpl	-0.5278217	0.01967461
Nop58	-0.5278991	0.002640916
Hsp90aa1	-0.5285458	0.004559047
Klhl21	-0.5298036	0.020200454
Rspo3	-0.5305875	0.003677673
Magoh	-0.5325781	0.021756697
Pfcp	-0.5343331	0.037114187
Casc5	-0.5346706	0.010635018
Katna1	-0.5392394	0.040535413
Haus1	-0.5416084	0.018415097
Ddx20	-0.5419028	0.007250069
Zwilch	-0.5423232	0.003516898
D8Erttd738e	-0.5444438	0.036293603
Ppa1	-0.545403	0.048289851
Isoc1	-0.5481145	0.0163981
Lin28a	-0.5484315	0.006155268
Cse1l	-0.5488957	0.000267407
Lss	-0.5497879	0.01150381
Mad2l1	-0.5499787	0.01318802
E2f4	-0.5500669	0.016680184
Cep76	-0.5505935	0.030346521
Noc3l	-0.5510514	0.008931862
Rap1b	-0.5510983	0.029783277
Ddx21	-0.551379	0.002062486
Zfp850	-0.5528011	0.034898561
Mrps22	-0.5561946	0.033198701
Ranbp1	-0.5567163	0.004602199
Umps	-0.5584059	0.006954703
Dut	-0.5585744	0.001840115
Mcm7	-0.5601798	0.000658803
Cep290	-0.5611988	0.0289515
Mif	-0.5644379	0.022222952
Usp36	-0.5669756	0.029260583
Ppp1r14b	-0.5692128	0.006049266

Hdgf	-0.5709078	0.000333168
Hist1h1e	-0.5717303	0.014219326
Cluh	-0.5718228	0.010217169
Cks2	-0.5722872	0.037976577
Gcsh	-0.5728595	0.001909046
Ahsa1	-0.574962	0.001385299
Pold1	-0.5749853	0.019814115
Sms	-0.5751655	0.003849886
Jmjd6	-0.5756012	0.007296571
Fbl	-0.5758456	0.00085362
Prps1	-0.5765977	0.040763638
Nop56	-0.5766727	0.037732378
Homer1	-0.5783565	0.035499608
Nhp2	-0.582946	0.00138054
Nasp	-0.5832083	0.0001497
Noc2l	-0.5835108	0.001151491
Rcc1	-0.5838483	0.01150381
Atad5	-0.5839931	0.000744992
Snrpd2	-0.5845768	0.049596743
Gsg2	-0.5887483	0.024804679
Mdk	-0.5925585	0.036779379
Maz	-0.5930999	0.031077508
Mrpl3	-0.5936589	0.010625531
Naf1	-0.5942042	0.005657614
Mid1	-0.5945538	0.010151093
Cenpn	-0.5953636	0.007380439
Pprc1	-0.595594	0.010734992
Dis3	-0.5961683	0.000516827
Naa10	-0.5967393	0.014832121
Ankrd27	-0.5970243	0.036430871
Rpp14	-0.5981019	0.032039462
Gnl3	-0.6009705	0.004260171
Pa2g4	-0.6011336	7.33916E-05
Kmt2b	-0.60144	0.035725263
Ddx39	-0.6014412	0.001368778
Nop10	-0.60184	0.006261636
Snopc4	-0.6024253	0.009725129
Spp12a	-0.6039032	0.01632944
Bop1	-0.6056497	0.006793952
Psat1	-0.6060635	0.00091311
E2f1	-0.6070543	0.01963896
Cap2	-0.6076249	0.000505951
Polr1b	-0.6098149	0.002530932
Naa50	-0.6103831	0.027909075

Nup35	-0.6118891	0.038555559
Timeless	-0.6145758	0.002158363
Pfas	-0.616097	0.000899475
Rps12	-0.6173728	0.03182126
Aste1	-0.618063	0.031421462
Zmym1	-0.6181706	0.010592254
Dtymk	-0.6182236	0.026420093
Kif15	-0.6183403	0.000279445
Haus3	-0.6188925	0.00930685
Mrpl52	-0.6198458	0.048700709
P2ry1	-0.6275068	0.026938192
Dusp18	-0.628064	0.012882798
Ccdc124	-0.6290632	0.02040009
Ell2	-0.6296718	0.018417458
Tk1	-0.6301929	0.037285962
Shmt2	-0.630889	7.98755E-05
Abcb10	-0.6330012	0.001300759
D030056L22Rik	-0.6356704	0.018113235
Akt2	-0.6369274	0.021342939
L2hgdh	-0.6389627	0.005507327
Rreb1	-0.6400927	9.95066E-05
Elac2	-0.6413754	0.002158363
Etl4	-0.6415104	0.001529963
2410002F23Rik	-0.6417525	0.01963896
Nkrf	-0.6418171	0.00353932
Mrpl47	-0.6420686	0.034731938
Lsm2	-0.6427002	0.012536752
9430015G10Rik	-0.6469001	0.028851222
Uchl3	-0.6487112	0.008916301
Dhodh	-0.6498502	0.024164708
Pole	-0.6524743	0.001188025
Nmral1	-0.653271	0.046458929
Rad51	-0.653761	0.004066553
Urb1	-0.6553645	0.006278841
Mob1b	-0.6574739	0.002452616
Nolc1	-0.6576932	8.64663E-06
Bckdhb	-0.6580894	0.022112927
Bag2	-0.6645741	0.02505003
Smyd5	-0.6656635	0.026721458
Ndufs6	-0.6699754	0.015364912
Itga2b	-0.6727368	0.020276582
Tsen2	-0.6775487	0.046466866
Edrf1	-0.6829337	0.00804993
Ppan	-0.6850071	0.035644434

Ptgr1	-0.6889211	0.022856973
Cdc6	-0.6929623	0.001740874
3110082I17Rik	-0.6937728	0.012238072
Diablo	-0.6948298	0.029975046
Rybp	-0.6951969	0.044775658
Dctd	-0.7007772	0.002543299
Mob3c	-0.7061391	0.029251299
Ippk	-0.7063486	0.011952822
Rab11fip1	-0.7064745	0.00787161
Mrpl22	-0.7076716	0.018435039
Sod2	-0.7101316	0.00083047
Srxn1	-0.7117592	0.020276582
Tlcd1	-0.7161115	0.034951497
Mtrr	-0.7165168	0.009427644
Uqcr10	-0.7168167	0.00711417
Heatr2	-0.7168978	0.006279683
Fancd2	-0.7181052	0.001233198
Tcof1	-0.718714	0.000177483
Trip13	-0.7194061	0.000102004
Rad54l	-0.7201007	0.006443575
Rpp40	-0.720515	0.008722369
Tal1	-0.7243217	0.035004677
Ccdc18	-0.7250853	0.022430669
Zfp3612	-0.7253139	0.001857072
Setd8	-0.7259416	0.000269964
Prkab1	-0.729529	0.000129675
Zbtb43	-0.7306579	0.038431642
Gins1	-0.7321125	0.009438331
Sf3b6	-0.7375405	0.022237244
Cep152	-0.7386925	0.035947492
Ung	-0.7392586	0.016867812
Snrpa1	-0.741571	0.000208255
Slc16a6	-0.7451852	0.010050368
Ska3	-0.746177	0.005971886
Egln3	-0.7466605	4.23684E-05
Bcor	-0.7480644	1.94473E-06
Zik1	-0.7482625	0.029931613
Srf	-0.7496534	0.003480838
Snhg5	-0.7520657	0.000951237
Uqcr11	-0.7552484	0.01150381
Ddx11	-0.7563069	0.004176478
Psmc3ip	-0.7568939	0.000419823
1110038B12Rik	-0.7581591	0.001273613
Helb	-0.7590714	0.020520561

Hsd17b7	-0.7599393	0.000674468
Prkcd	-0.7609372	0.04692825
Smim3	-0.7616062	0.011321729
Notch2	-0.7648085	0.001607176
Hmgn2	-0.7648535	0.003655177
Lsm5	-0.7653828	0.020768793
Tfb2m	-0.7675262	0.016153005
Anapc11	-0.7680224	0.034225692
Ppih	-0.7682877	0.016367779
H2afx	-0.7685989	0.000594153
Fancb	-0.7713014	0.041391858
Gyk	-0.7715793	0.046836397
Polr2i	-0.7720022	0.01984889
Depdc1b	-0.7720366	0.001707355
Zcchc10	-0.7745844	0.005241081
Snrpg	-0.7818499	0.04215328
Klhl12	-0.7832182	0.001815011
E2f8	-0.78417	0.000180926
Epb4.1	-0.7849334	1.37786E-05
Wbscr16	-0.79124	0.018213973
Fth1	-0.7925434	0.000310477
Troap	-0.7972141	0.023151903
Hspe1	-0.7988842	0.01963896
Rnd2	-0.8016923	0.022634645
Ern1	-0.8027457	0.004274098
Mtx3	-0.8052675	0.042241708
Suv39h2	-0.8053629	0.000264292
Tcf7	-0.8056699	3.70941E-05
Lyar	-0.806388	5.67508E-06
Smtnl2	-0.8080904	0.013224797
Tnfaip8	-0.8116157	0.001896077
Peo1	-0.8136152	0.007218947
Speg	-0.81617	0.021756697
Fut8	-0.8166749	0.000104545
D2Wsu81e	-0.8168487	0.008155523
H2afz	-0.8234957	3.07822E-08
Laptm5	-0.8267113	0.043365121
Ubxn2a	-0.8271654	0.000222764
Stra13	-0.8316425	0.012298527
Rrp12	-0.8351638	0.000109874
Gcnt1	-0.8393189	2.35311E-05
Tsc1	-0.8417155	0.00012396
Trmt61a	-0.8425698	0.000893326
Polr2f	-0.8473131	0.007191282

Utp23	-0.8561307	0.010212308
Mrto4	-0.8586158	0.000100664
Fads2	-0.8661943	4.81338E-06
Tm7sf2	-0.8688088	0.025432283
Nbeal2	-0.8716841	0.006623268
Ska1	-0.8730391	0.036533027
Traip	-0.8757748	0.020692508
Psph	-0.8766564	0.006842544
Inip	-0.8770961	0.001718342
Ormdl1	-0.8771678	0.00814927
Srl	-0.8787499	0.023134393
C530008M17Rik	-0.8817309	0.02435718
Hist1h1b	-0.883334	5.46909E-05
Caprin2	-0.8843534	0.005657628
Rbmx	-0.8861848	0.000459628
Grap2	-0.8868077	0.024330801
Sssca1	-0.8898676	0.000505951
Gas5	-0.8931038	0.001005654
Melk	-0.8938092	0.000748058
Idi1	-0.8942903	0.000625845
Bcat1	-0.9044063	0.006141257
Mdm1	-0.9053117	7.9056E-05
Rps6ka6	-0.9072983	2.512E-06
Prmt1	-0.9141192	0.000276921
Fkbp11	-0.9174265	0.030220191
Slc19a1	-0.9234819	0.012924042
Ppil3	-0.9241589	0.033070183
Gtf3c5	-0.9250698	8.09209E-08
Hist1h2bb	-0.9256411	0.034731938
Spc24	-0.9296335	9.00545E-05
Itga4	-0.9299064	0.000123511
Hist1h2be	-0.9304375	0.022222952
Pola2	-0.9380601	1.61723E-05
Ahi1	-0.9508248	2.51545E-05
Acy1	-0.9528935	0.043805059
Ctla2a	-0.9543965	0.049700762
Cenpp	-0.9595026	0.01722529
Gpc6	-0.9623014	0.010298045
Shroom3	-0.9652569	0.038151613
Zfp692	-0.9781341	0.00385429
Tmem158	-0.9823048	0.025921359
Cited2	-0.9926737	1.75743E-06
Taf1d	-0.9953624	0.036966046
Acot11	-1.0008928	0.00777375

Lsm7	-1.0009126	0.004305268
Slc7a5	-1.0036	2.17805E-06
Tmem86b	-1.0039618	0.017467063
Ifi30	-1.0044766	0.048700709
Psd3	-1.0174544	0.03534982
Wdsub1	-1.0239369	0.048950469
1810032O08Rik	-1.0328231	0.029931613
Hist1h4d	-1.0383794	0.005507327
Dctpp1	-1.0393153	0.000317381
Plscr1	-1.039389	0.004614916
Pmp22	-1.0535001	0.009847584
Mgat4a	-1.0568436	0.007776813
C77080	-1.0585692	0.000189306
Snhg3	-1.0648738	0.010269121
Sec61g	-1.0666418	0.017108692
Psmg4	-1.0676331	0.046324916
Sdc1	-1.0753188	0.000999753
Fzd2	-1.0778418	0.027968234
Lhpp	-1.0879831	0.02047849
Gse1	-1.0939363	2.31344E-11
Zfp930	-1.0940181	0.000645702
Lsm3	-1.0963022	0.004523766
Tagln	-1.1030145	0.034761101
Car2	-1.1035294	0.035986008
Kcnj5	-1.1045913	0.000544414
Med30	-1.1093461	0.019362238
Dsp	-1.1110028	0.009917825
Lpar1	-1.1156382	0.033861499
Kalrn	-1.1171644	1.07783E-06
Ccdc134	-1.1231804	0.020684784
Xk	-1.1366802	0.007849919
Dyrk2	-1.1426776	0.009743851
Irs1	-1.1430887	0.002965951
Kctd15	-1.1434019	0.028175567
Lyl1	-1.1442502	0.000958535
Apitd1	-1.1442509	0.00232128
Ank3	-1.1478545	5.47484E-07
Etv2	-1.1482494	0.003357049
Arhgap6	-1.1576452	1.26249E-05
Tpm2	-1.1599192	0.005227615
Cth	-1.1612232	0.032836443
Mtfp1	-1.162728	0.010298045
Chchd1	-1.1640701	0.042687922
Fam109b	-1.1687442	0.034422349

Snrnp25	-1.1795927	8.64663E-06
Tfrc	-1.1817508	1.42962E-15
Fzd10	-1.1850427	0.012027882
Robo2	-1.1976152	0.027968234
Rab27b	-1.2026754	0.000262575
Hist1h3e	-1.207497	0.002028412
Hist1h2bm	-1.2087727	0.000166046
Pde4dip	-1.2117548	2.22157E-08
Myl9	-1.2154017	0.008966617
Col5a1	-1.2365169	0.024124732
Nfix	-1.2439774	0.028851222
Plek	-1.2460721	0.029677111
Hist1h2ac	-1.2654033	0.048005393
Sh3bp1	-1.2655429	3.82269E-06
Slc35f2	-1.2714037	0.004479282
Med12l	-1.2776314	5.18855E-08
Eno3	-1.2819185	0.00053602
Gria2	-1.2929235	0.012739783
Prtg	-1.2975163	3.8001E-10
Samd14	-1.3126027	0.034398076
Gfra2	-1.3188517	0.001212132
Hist2h2ac	-1.3249293	0.043805059
Hist1h2bl	-1.3323982	3.94278E-06
Hist1h4c	-1.3424522	0.036779379
Lgals9	-1.3443204	0.001153782
Phgdh	-1.3465429	1.0238E-06
Hist1h4a	-1.3568512	0.040921913
Hspb8	-1.3581106	0.047653617
Hist1h2bf	-1.3608963	9.84748E-05
Lama1	-1.3748046	0.010539709
Ptpre	-1.3799639	6.33328E-07
Mpl	-1.383543	0.00145656
Gfi1	-1.3873919	0.029781297
Tenm3	-1.3946725	0.036732568
Klf8	-1.398728	1.0931E-09
Cyp26b1	-1.4037073	0.010353402
Hist1h2ak	-1.4072807	0.000275295
Pitx2	-1.410157	0.003136119
Dyrk3	-1.4106397	0.004579839
Nap112	-1.4284217	0.041255062
Ston2	-1.4291669	0.000106664
Hist1h3g	-1.4334317	0.001909046
Ifitm1	-1.4344149	5.82107E-05
Adamts9	-1.4390785	0.020080206

Mrvi1	-1.439407	0.0163981
Sfrp2	-1.4502113	0.034731938
Lrr1	-1.45511	0.004465313
Mpo	-1.4663655	0.01179793
Ryr2	-1.4690604	0.016935002
Unc5c	-1.4771939	4.14642E-15
Egfl8	-1.4885643	0.048391189
Fam117a	-1.4937007	0.00425314
Hist1h2bh	-1.4980855	4.37625E-05
Syt7	-1.5153341	0.017781453
Gpc3	-1.5323008	7.16894E-09
Sall3	-1.5370094	1.50775E-17
Wnt5a	-1.5468432	0.000760804
Hist1h3i	-1.5483412	0.026721458
Plcx1	-1.5523584	0.038153598
Hist1h2bp	-1.5681605	0.009899788
Hist1h2bk	-1.5735921	0.00043405
Snord99	-1.5909402	0.016290154
AI467606	-1.5969964	0.022946391
Ptprj	-1.6077737	1.0931E-09
Ctse	-1.6078993	0.002431662
Prkca	-1.6098811	3.36158E-10
Rgs10	-1.6222642	0.013254674
Wisp1	-1.63618	0.000674468
Hist1h1a	-1.6408645	0.00010791
Dlg3	-1.6444894	0.038467385
Krt19	-1.6450808	0.004714719
Pappa	-1.6741823	0.020220736
Cnn1	-1.6778678	0.028766399
Dapp1	-1.7240331	1.26418E-07
Cend2	-1.7249129	8.48488E-05
Sdc2	-1.7252224	0.001295174
Slc30a3	-1.7763864	0.020783337
Hist1h2ai	-1.8043194	0.048613885
Nrgn	-1.8055743	0.009964598
Aif1l	-1.8075623	0.000117149
Atp2a3	-1.8125327	2.57553E-06
Rps6kl1	-1.8244938	0.049403296
Gm16982	-1.848031	0.031813334
Tmem62	-1.8548086	0.013302571
Thsd4	-1.867671	0.002237663
Col1a2	-1.8752501	0.000867063
Kcnj2	-1.8778166	0.009381743
Itgb3	-1.904678	1.03962E-06

Snord47	-1.908005	0.035065481
Hist1h4k	-1.9096921	0.00594326
Gng7	-1.9207414	0.01967723
Ackr3	-1.9236598	0.001112783
Fam46a	-1.9265152	0.007309261
Cdh11	-1.9347074	0.003681232
Acta2	-1.9431264	0.001082949
Igsf11	-1.9730923	0.0032421
Hbb-y	-1.9748359	4.58395E-05
Ubxn11	-1.983615	0.006842544
Ikzf2	-1.9955979	8.66601E-16
Epcam	-1.9977628	0.010155013
Was	-1.9990616	8.50094E-05
Acat3	-2.0060747	0.027344644
Mcoln3	-2.0135873	0.03269587
Hist1h4j	-2.0168569	0.037114187
Gli2	-2.0256238	0.001703197
Smyd1	-2.0480579	0.018380174
Pde11a	-2.0583309	0.028033733
Map7d2	-2.0666869	0.032464685
Wwc1	-2.0764389	0.000471852
Hba-x	-2.0906682	3.15899E-07
Irs2	-2.0961118	5.87776E-20
Pdgfrb	-2.1019287	0.007434748
Ptpn13	-2.1059753	4.78986E-08
Nfe2	-2.1065983	4.46548E-08
Smim1	-2.1151748	0.014903031
Fgf10	-2.1307131	1.51684E-05
Wnt4	-2.1362897	0.001401918
Snord15b	-2.157675	0.00934557
Sytl5	-2.1590435	0.000440282
Syne4	-2.1602438	0.006038733
Lrp2	-2.1683193	0.000121691
Kcnd3	-2.1909069	0.006903889
Kif26b	-2.2020228	0.034731938
Tspan32	-2.2086198	8.02448E-06
Gfi1b	-2.2107003	1.69686E-06
Cgn	-2.2139564	0.002217795
Chpf	-2.2346358	0.021439509
Col12a1	-2.2377922	1.30785E-05
Mir1949	-2.252971	0.006517304
Reep6	-2.2591005	0.000441881
Gucy1a3	-2.2596713	3.05581E-09
Fam110c	-2.2660124	0.047497123

Pkd113	-2.2721338	0.024648589
Col8a1	-2.275621	0.004586186
Ikzf1	-2.2786084	1.54656E-16
Phlda2	-2.2808251	0.000544414
Sdk2	-2.3005852	0.001789848
Cort	-2.3033757	0.043196035
F3	-2.3463844	0.002491942
Ildr2	-2.3554778	0.025410734
Cnksr2	-2.4145604	0.001740874
Adamts3	-2.4415041	1.14347E-16
Slit3	-2.4443195	0.016761722
Ccl17	-2.4618337	0.005656976
Scube2	-2.4633338	1.24981E-05
P2rx1	-2.4648688	4.24094E-06
Plbd1	-2.4842592	0.043805059
Nog	-2.4867721	0.030749983
Evx1	-2.5070756	0.00710432
Pard6b	-2.5132174	7.02155E-11
Dlk1	-2.5174664	0.011179574
Lypd6	-2.527006	0.012079092
Slc16a10	-2.5476922	1.04806E-05
Igfbp2	-2.5554076	1.85263E-05
Epb4.2	-2.5598202	1.80207E-05
Krt8	-2.5741038	1.01582E-06
Terc	-2.5834174	0.019295424
Hoxd1	-2.621979	8.15008E-07
Myl4	-2.630741	0.007701513
5330426P16Rik	-2.6446846	0.028999329
Adamts15	-2.6619076	0.001835464
Klhl34	-2.6888257	0.024330801
Snord65	-2.6966047	0.013699384
Zic3	-2.7075066	0.010077455
Chac1	-2.7158122	0.03425189
Car12	-2.7161838	0.0125579
Runx1	-2.7256084	1.65762E-27
Gpr56	-2.7306544	6.13521E-12
Lypd1	-2.7503944	0.029587149
Bdnf	-2.7569741	0.017067973
Cemip	-2.7597959	0.009725129
Psd2	-2.7685594	0.040231909
Bhlhe22	-2.7788676	0.045899837
Nkx1-2	-2.8116603	4.82819E-06
Krt18	-2.8300695	1.94958E-05
Fgf15	-2.8448297	0.000744992

Ripk4	-2.8502852	0.035644434
Acta1	-2.8507773	0.006706841
Mfsd2b	-2.8581726	3.30438E-10
Hbb-bh1	-2.8585705	2.13735E-10
Nnat	-2.870562	0.009436605
1700024P16Rik	-2.9094172	0.046185933
Trem12	-2.9188545	0.016448242
Il18rap	-2.9367721	0.025586714
Cdh22	-2.9609251	0.04793262
Lgr5	-2.9755872	4.11712E-26
Rgs4	-3.045216	0.004274047
Bmp8a	-3.0462458	0.000513324
Enpp1	-3.0546272	4.11656E-06
Asic4	-3.0661955	0.008931862
Col11a2	-3.1009018	0.026664828
Igfbp5	-3.1027981	5.0016E-11
Scube3	-3.1383901	0.00062887
Crabp2	-3.2575996	0.006155268
Kcnk1	-3.2686447	3.72197E-05
Rpph1	-3.281995	2.1394E-05
Lmod1	-3.2934985	0.027317316
Cacna1d	-3.3339941	0.015484623
Dpf3	-3.3366168	0.00736531
Gm14204	-3.3480536	0.003222281
Cdh3	-3.3515719	4.85866E-06
Svep1	-3.3665107	0.024600296
Plac8	-3.3850148	0.035162852
Ppp1r14a	-3.3875344	0.02733033
Snord11	-3.4033326	0.03447524
Col2a1	-3.4043366	0.007702911
Krtap5-4	-3.4274958	0.013850109
Tenm4	-3.4333517	0.000392286
Postn	-3.4429613	8.23112E-10
Myb	-3.4543925	8.33645E-09
Inhba	-3.4548191	0.000713988
Tgfb3	-3.4614945	0.004896411
Slc32a1	-3.4787561	0.01146119
Twist2	-3.486109	0.013411972
Vgll3	-3.5369871	4.22616E-07
Actc1	-3.5579124	7.50346E-08
Bnc2	-3.5880639	0.009472726
Reln	-3.6170028	3.50005E-25
Krt14	-3.6405079	0.039694071
Sycp2	-3.6541894	0.034731938

Olf893	-3.6656714	0.044066221
Coll1a1	-3.6976688	4.95442E-05
Slc7a8	-3.7032242	5.53584E-19
Snord49b	-3.7060819	0.016344227
5830432E09Rik	-3.7217186	0.018434404
Mir3473	-3.7421314	0.043179572
1700019A02Rik	-3.8088791	0.011713162
Loxl1	-3.8129879	0.000497308
Mfap4	-3.8436132	1.62745E-06
Slc14a1	-3.9021836	0.032075036
Ms4a4d	-3.9106388	0.009878012
Arpp21	-3.9845324	0.013484556
Tuba8	-4.0014776	0.004465313
B4galnt3	-4.0405548	0.017018905
Mir1188	-4.0457792	0.047141713
Cbln1	-4.0779217	1.74552E-06
Bzrap1	-4.0941697	0.025511069
Mycbpap	-4.1183436	0.013319538
Tacstd2	-4.1381791	0.016813797
Mir6516	-4.1802058	0.000180383
Nrip3	-4.2215567	6.59402E-12
Pdzk1ip1	-4.2568504	4.63652E-06
Ajap1	-4.2816439	0.043805059
Ccl3	-4.4113143	0.007390122
Muc13	-4.4403089	4.55768E-05
Cobl	-4.4440277	0.012298527
Dlx5	-4.4620843	0.022676496
Col8a2	-4.5076296	0.028016048
D830026I12Rik	-4.542468	0.00273577
Smoc2	-4.5827836	0.049940314
Mir6940	-4.5833125	0.020094533
Sema5b	-4.5908909	0.000185175
Ndufa4l2	-4.6090649	0.00112525
Slc1a6	-4.6456547	0.001159997
Pgm5	-4.6485806	0.026721458
Gabra4	-4.7118276	0.042610543
Alx3	-4.8471877	0.01150381
A4gnt	-5.1071371	0.03788653
Vat1l	-5.1080698	0.0032421
Slitrk2	-5.1635952	0.004155895
Pip5k1l	-5.1675678	0.014014826
Mir1940	-5.2146295	0.036533027
Dppa5a	-5.2384808	2.24686E-06
Tph1	-5.285813	0.017552353

Bmp7	-5.3633196	0.00053602
Atp6v0d2	-5.5623301	0.000386004
Pitx1	-5.5980066	0.013302571
Olfml1	-5.6903819	0.001273613
Prr15l	-5.7339197	0.017886152
BC027072	-5.7931802	0.011830787
Zfp641	-5.8463841	0.048973333
Gata1	-5.9307033	1.14278E-08
Fgf3	-5.9991188	1.15752E-07
Rnase6	-6.0909008	0.04215328
Lum	-6.1112301	0.020767104
Vmn2r2	-6.1502481	0.026296039
Atp6v0a4	-6.270693	0.024844203
Clec18a	-6.5983151	3.59311E-05
Elfn2	-6.6897525	0.001713009
Cd164l2	-7.1662694	0.003073471
Wfdc17	-7.1680774	0.000253078
Fstl3	-7.2822667	0.001909046
Tmem54	-7.5876681	0.000153509
Orm1	-7.628743	0.000867063
Sema4f	-8.0860262	0.002174643